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Identification of Sockeye Salmon (*Oncorhynchus nerka*) Stocks in the Stikine River Based on Egg Size Measurements

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Craig, P. C. 1985. Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in the Stikine River based on egg size measurements. Can. J. Fish. Aquat. Sci. 42: 1696–1701.

The Stikine River in southeastern Alaska supports two principal stocks of sockeye salmon (*Oncorhynchus nerka*). An expedient means of separating these stocks when they enter freshwater is by egg size and egg mass measurements. Eggs of sockeye that spawn in the Tahltan Lake system are smaller than sockeye spawning elsewhere in the Stikine drainage, a difference supported by an independent stock identification method (scale pattern analysis). Tahltan females have only slightly developed ovaries (1–3% of body weight) and small eggs (2.0–3.5 mm) when they enter freshwater; they enter the river earlier (2–3 wk) and travel farther upstream to spawn than non-Tahltan sockeye. The latter group has more developed ovaries (5–16% of body weight) and larger eggs (3.5–6.2 mm) when they enter the river. Thus, an assessment of egg sizes of sockeye caught in the river can provide *on-site* information about stock composition and run timing. These data and escapement counts at Tahltan Lake indicate that the total escapement to the Stikine in 1983 was 61 000 to 71 000 sockeye of which 45–53% were Tahltan stock and 47–55% spawned eleswhere in the drainage.

Deux importants stocks de saumon rouge (*Oncorhynchus nerka*) peuplant la rivière Stikine, au sud-est de l'Alaska. La quantification de la taille et de la masse des œufs sert de moyen pratique pour l'identification de ces stocks quand ils entrent en eau douce. Les œufs du saumon rouge qui fraie dans le système du lac Tahltan sont plus petits que ceux du saumon rouge se reproduisant à d'autres endroits du bassin versant de la Stikine : cette différence est étayée par une méthode indépendante d'identification de stock (analyse scalimétrique). Les ovaires des femelles du système du Tahltan sont peu développés (de 1 à 3 % du poids corporel) et leurs œufs sont petits (2,0–3,5 mm) quand elles entrent en eau douce, de 2 à 3 sem avant les saumons rouges qui frayent ailleurs; de plus, elles remontent plus loin en amont que ces derniers. Les ovaires de ceux-ci sont plus développés (de 5 à 16 % du poids corporel) et leurs œufs sont plus gros (3,5–6,2 mm) quand ils entrent en milieu fluvial. Une évaluation de la taille des œufs du saumon rouge capturé dans la rivière peut donc fournir des données instantanées sur la composition du stock et le moment de la remonte. Ces données, en plus des dénombrements de l'échappée dans le lac Tahltan, révèlent qu'en 1983, l'échappée totale dans la Stikine variait de 61 000 à 71 000 saumons rouges dont 45–53 % représentaient le stock du Tahltan et 47–55 % se reproduisaient ailleurs dans le bassin versant.

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The Stikine River is an international drainage which originates in northern British Columbia and flows into the Alaskan panhandle near Wrangell, Alaska (Fig. 1). It supports a population of sockeye salmon (*Oncorhynchus nerka*) that spawns in Canada but is harvested primarily by Alaskan fishermen in coastal waters. Canadians also harvest some of these sockeye at upriver commercial and Native food fisheries. In past years it was assumed that Tahltan Lake was the major producer of sockeye in the Stikine drainage, with as much as 90% of the population spawning there (Bergmann 1978). Recent evidence, however, indicates that more sockeye than previously thought spawn elsewhere in the drainage; spawning populations have been located in the turbid, glacial-fed mainstem of the Stikine as well as in several tributaries: the Iskut, Chutine, and Christina rivers (McCart and Walder 1982).

Differentiation between Tahltan Lake and non-Tahltan Lake stocks of sockeye in the Stikine River has been recently achieved using scale patterns (McCart and Walder 1982; Oliver 1983; McPherson 1983; Walls 1984; Lynch and Eggers 1985) and electrophoresis and parasite markers (B. Riddell and C. Wood, Department of Fisheries and Oceans (DFO), Pacific Biological Station, Nanaimo, B.C. V9R 5K6, pers. comm.). Scale pattern analyses indicate a relatively large stock of non-Tahltan sockeye in the Stikine, annually comprising 38–69% of the sockeye produced in this drainage.

The present report describes an additional and more expedient technique which allows stock separation based on egg size and egg mass patterns. The utility of this technique is then demonstrated by calculating escapements and run timing of sockeye stocks in the Stikine River. For convenience, the two main groups of Stikine sockeye are referred to in this report as "Tahltan stock" and "river stock," recognizing that the latter may consist of a variety of non-Tahltan spawning locations.

Methods

The Canadian in-river fishery (Great Glacier Fish Company) is located in the Stikine River near the Alaska–Canada border

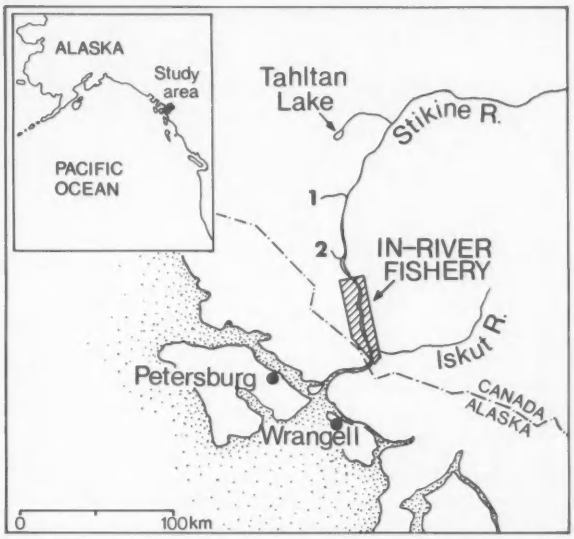

FIG. 1. Pacific Northwest showing Stikine River and locations of the Canadian in-river fishery, Chutine River (1), and Christina River (2).

(Fig. 1). From 4 July through 23 August 1983, a subsample of the weekly gillnet catch of this fishery was examined by a joint team of Alaskan and Canadian fisheries technicians. In addition to weight (to the nearest 5 g) and length data (to the nearest 1 mm), fish gonads were weighed (to the nearest 0.1 g) and egg diameters were measured (10 eggs were aligned and the distance from the outer edge of the first egg to the outer edge of the tenth egg was measured to the nearest 1 mm and then divided by 10). Scale pattern analyses and aging for the same specimens were provided by G. Oliver (Alaska Department of Fish and Game (ADFG), P.O. Box 20, Douglas, AK 99824).

Catch statistics for the Canadian in-river commercial and Native food fisheries, and the escapement and egg sizes of sockeye passing through the Tahltan Lake counting fence were provided by S. Johnson (DFO, 122 Industrial Rd., Whitehorse, Yukon Territory Y1A 2T9).

In 1984 the in-river commercial fishery did not operate, but the joint Alaskan–Canadian team measured egg sizes of sockeye caught in a test fishery located 25 km upstream of the Stikine River mouth (S. Johnson, DFO, pers. comm.; Lynch and Eggers 1985). This gillnet fishery operated almost daily from 19 June to 17 August 1984.

Results

Stock Separation Using Egg Sizes

Female sockeye caught in the Canadian in-river fishery averaged 564 mm in length (mid-eye to tail fork) and 3.0 kg in weight (Fig. 2). Although length and weight distributions were unimodal, there was a bimodal pattern of egg sizes in samples collected early in the season; females contained either small eggs about 2.2–3.2 mm in diameter or large eggs about 3.8–5.4 mm (Fig. 3A). This pattern persisted throughout the July–August fishery for sockeye (Fig. 3C), suggesting that little further development of the small eggs occurred at this time and location because few fish with intermediate-sized eggs were present. A more symmetrical bimodality of egg sizes in the total fishery

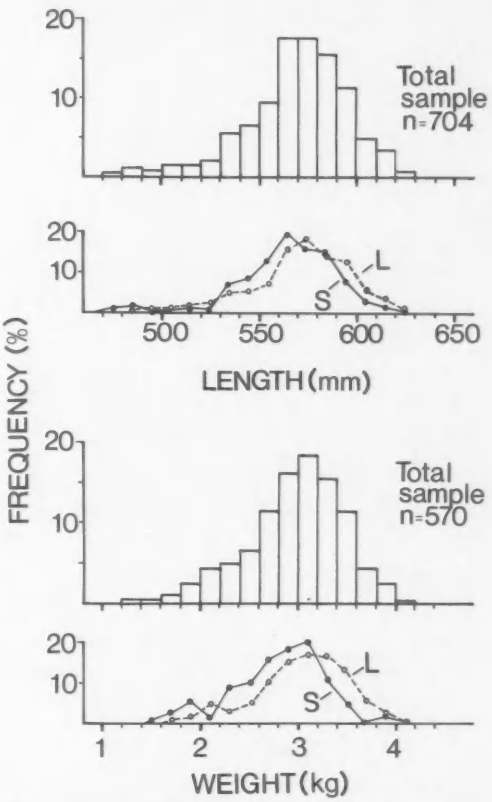

FIG. 2. Length and weight frequencies of Stikine sockeye females in the in-river fishery, July–August 1983. Length, mid-eye to tail fork; L, large-egg females; S, small-egg females.

was presumably not obtained in 1983 because early-run sockeye probably had small eggs but were not sampled (discussed later). In 1984 when samples were collected over a more complete time period (19 June – 17 August), a bimodal and more symmetrical pattern of egg sizes was recorded (Fig. 3D).

The validity of separating the 1983 sample of female sockeye into two groups based on egg sizes was supported by a gonadosomatic index (% ovary weight/body weight) which separated the fish into distinct groupings (Fig. 4). Fish with small eggs contained relatively underdeveloped gonads (1–3% body weight), while those with large eggs had well-developed gonads (5–16% body weight). In the commercial fishery, the difference between the egg masses of large- and small-egg fish was so apparent that roe from the former were kept for market but the latter were discarded.

Although the egg sizes of a few females were intermediate between the large and small egg groupings, virtually all of these fish could be assigned to one pattern or the other based on their gonadosomatic index value. A gap separating the two groups occurred at about 3.4 mm (early July) to 3.6 (mid-August) for sockeye in the lower Stikine River in 1983. Together, the two variables of egg size and gonadosomatic index provided the best separation of sockeye groups (Fig. 5) and were used to define "small-egg fish" and "large-egg fish" as used in this paper.

These data indicate that two different groups of fish were present in the Stikine River: one more mature than the other,

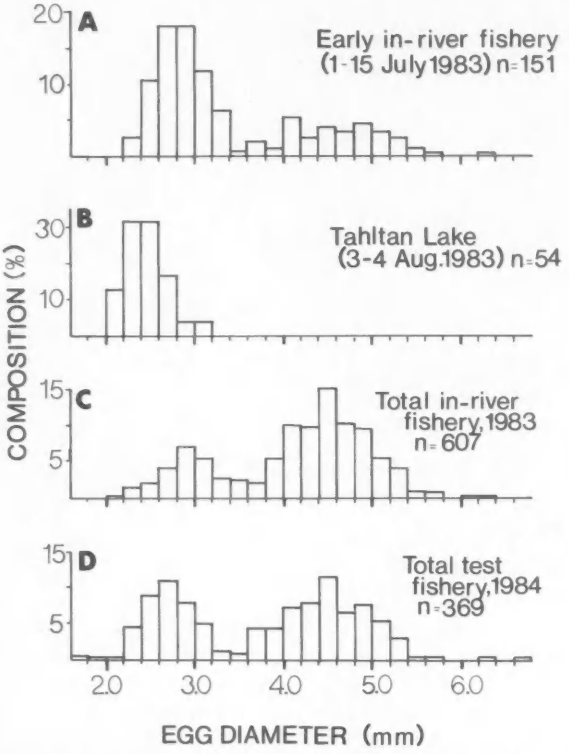

FIG. 3. Egg sizes of sockeye caught in the Canadian in-river fishery and at Tahltan Lake (1983), and in the Alaskan test fishery (1984).

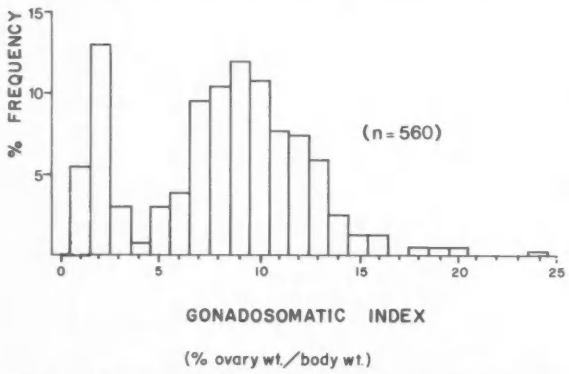

FIG. 4. Frequency distribution of the gonadosomatic index of female sockeye caught in the Canadian in-river fishery, July–August 1983.

perhaps because the less mature group spawned later or had farther to travel to reach its spawning grounds. Differences between the two groups in migration timing (discussed later) also support the contention that two stocks exist. The strongest evidence is, however, that Tahltan females caught at the counting fence at Tahltan Lake all had small eggs (Fig. 3B). Because it seems unreasonable that large-egg fish in the lower Stikine would decrease their egg size as they migrate upriver, the small-egg fish in the commercial fishery must have included the Tahltan stock. Whether some small-egg fish also represented

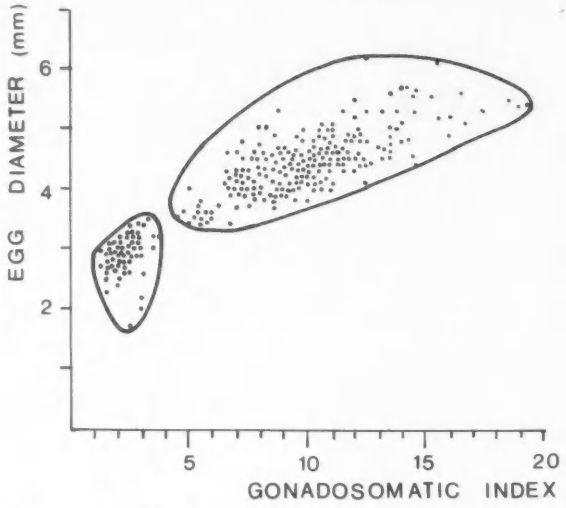

FIG. 5. Relationship between egg size and gonadosomatic index, July–August 1983.

late-spawning non-Tahltan fish is not known. For the present, I have assumed that small-egg fish represented only the Tahltan fish.

The egg sizes of known Tahltan fish measured at Tahltan Lake on 3–4 August 1983 were somewhat smaller than would be expected based on egg size measurements of small-egg fish in the lower Stikine in early July (Fig. 3B). Reasons for this apparent difference between the two sample means (0.4 mm) are not known, although it should be noted that measurements at the Tahltan fence were made only once during the early portion of the run whereas a seasonally stratified sample would be preferred.

Body sizes of large-egg fish females were significantly larger than small-egg females ($P < 0.025$), but the differences were small, thus precluding the use of size measurements for stock separation. Large-egg fish averaged 1.3 cm longer and 0.3 kg heavier than small-egg fish (Fig. 2). These differences suggest that a comparison of body size and egg size for the *total* (i.e. combined) Stikine sample might produce a statistically significant relationship (the larger fish had larger eggs), but this relationship would be misleading because the total sample consisted of two separate stocks at different stages of maturity.

Comparison with Scale Patterns

Scale patterns have also been used to separate Tahltan and river stocks of sockeye in the Stikine River. River sockeye (age 1.3) generally have up to 10 scale circuli present during their first freshwater year whereas Tahltan sockeye of the same age generally have 11 or more circuli (McCart and Walder 1982; Oliver 1983; McPherson 1983; Walls 1984; Lynch and Eggers 1985). Stocks separated via egg sizes showed corresponding differences in scale circuli (Fig. 6). Sockeye taken in the fishery and designated as river stock based on their egg sizes had fewer circuli than did the presumed Tahltan stock, although ranges of cirlculi were broader than those estimated by scale analysis alone. Of the 471 sockeye for which both egg size and scale data were available for individual specimens, there was an 88% agreement in stock designation by the two techniques in 1983.

TABLE 1. Weekly proportions and catch per unit effort (CPUE) of small-egg (S) and large-egg (L) fish in the Canadian in-river fishery, 1983.

Week ending	Subsample			Commercial catch			Commercial CPUE		
	n	%S	%L	Total	S	L	Effort[a]	S	L
June 18	—	100[b]	0[b]	45	45	0	14	3	0
25	—	100[b]	0[b]	179	179	0	14	13	0
July 2	—	95[b]	5[b]	1 060	1007	53	27	37	2
9	146	85	15	2 240	1904	336	36	53	9
16	99	59	41	2 912	1718	1194	40	43	30
23	94	37	63	1 919	710	1209	36	20	34
30	68	23	77	3 312	762	2550	36	21	71
Aug. 6	85	11	89	2 315	255	2060	36	7	57
13	105	6	94	1 312	79	1233	24	3	51
20	72	10	90	235	24	211	16	2	13
27	38	5	95	114	6	108	8	1	14
Sept. 3	—	2[b]	98[b]	146	3	143	24	0	6
10	—	0[b]	100[b]	78	0	78	24	0	3
17	—	0[b]	100[b]	3	0	3	40	0	0
Total	707	36	64	15 870	6692 (42%)	9178 (58%)			

[a]Number of days fished × number of fishermen.
[b]Assumed; biological sampling not performed.

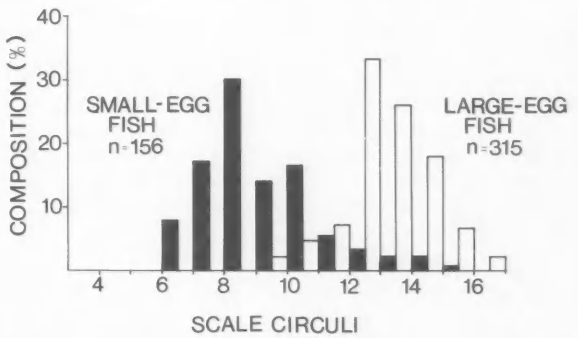

FIG. 6. Number of scale circuli in the first freshwater year for stocks identified on the basis of egg size, 1983.

There was no obvious pattern to the mismatches; further study would be needed to assess which technique provides more accuracy. For example, studies in 1984 revealed that some sockeye with Tahltan-like scale patterns were found spawning in non-Tahltan locations in the drainage (G. Oliver, ADFG, pers. comm.), thereby complicating the interpretation of scale pattern data.

Stock Composition and Timing of Stock Runs

The commercial catch of the Canadian in-river fishery was divided into stock components from the proportions of small- and large-egg females caught during each week of the fishery. In 1983 the in-river catch of 15 870 sockeye consisted of 42% Tahltan stock and 58% river stock (Table 1). Because these calculations were based on proportions of females in catches, it was necessary to assume that the sex ratio of the Tahltan stock was similar to that of the river stock.

The catch per unit effort (CUPE = number of sockeye caught per fisherman-day in the commercial fishery) data indicate that the Tahltan run occurred 2–3 wk earlier than the river run (Fig. 7 and 8). However, another line of evidence suggests that the Tahltan fish began migrating even earlier. Tagging data indicate that sockeye take an average of 30 d (n = 8, range 21–38 d) to travel from the lower Stikine River to the Tahltan Lake counting fence (B. Lynch, ADFG, Peterburg, AK, pers. comm.). Using this rate of travel, it is possible to estimate when fish caught at the Tahltan fence should have passed the downstream commercial fishery. Because the first third of the Tahltan run passed through the fence before 23 July (Fig. 7) and there were only two short unproductive fishery openings before 2 July, an early pulse of Tahltan sockeye may not have been subject to much commercial exploitation in the river.

Stock Escapement

Two kinds of escapement in the Stikine River are (1) *border escapement*, the number of sockeye entering the river and migrating into Canada, and (2) *spawning escapement*, the number of sockeye that escape all fisheries and reach their spawning grounds.

Spawning escapement at Tahltan Lake is known because a counting fence is maintained there. Spawning escapement elsewhere in the drainage can be estimated from the relationship

$$\text{ERS} = \text{CRS} \times \text{ETS}/\text{CTS}$$

where ERS is the escapement of the river stock, CRS is the commercial catch of river stock, ETS is the escapement of the Tahltan stock, and CTS is the commercial catch of Tahltan stock. This relationship assumes that all Stikine stocks are exploited in proportion to their runs; however, information on the run timing presented above suggests that the commercial fishery may not have exploited the first third of the Tahltan run. Steady fishing throughout July and early August (Table 1) suggests that the remainder of the Tahltan and river stocks was exploited in a proportional manner. Therefore, instead of using only the total Tahltan escapement of 21 256 sockeye in the

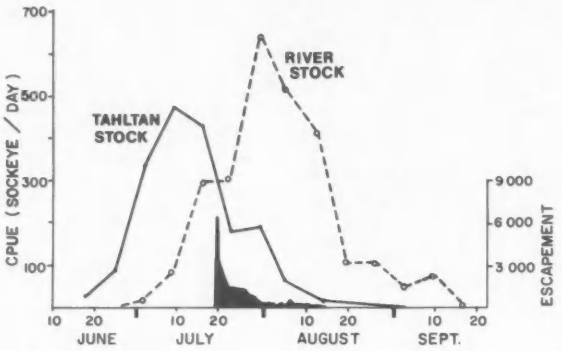

FIG. 7. Run times of sockeye stocks in the lower Stikine fishery, and the escapements at the Tahltan Lake counting fence (in black), 1983.

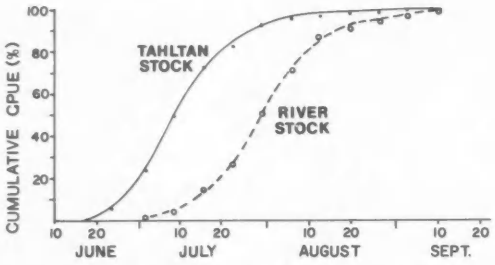

FIG. 8. Cumulative border escapement of sockeye, 1983.

above relationship, an escapement range with a lower and upper bound of 14 000 and 21 256, respectively, is more appropriate. Because proportions of river and Tahltan stocks in the fishery were 58 and 42%, respectively, there would have been 19 000 to 29 000 river spawners in 1983. The total 1983 Stikine spawning escapement would be between 40 000 and 50 000 sockeye. However, there are at least several factors that suggest these estimates may be biased downwards, as follows.

Iskut River factor

The Canadian in-river fishery captures fish almost exclusively in the Stikine River at locations upstream and downstream of the Iskut River where sockeye are known to spawn. Thus, commercial catches below the Iskut confluence sample the border escapement, while those above the Iskut sample the border escapement minus any fish that entered the Iskut River.

I obtained an estimate of the number of spawners in the Iskut River by comparing ratios of small- and large-egg fish caught above ($n = 76$) and below ($n = 70$) the Iskut River in the commercial fishery, 4–5 July 1983. Presumably, any sockeye entering the Iskut River would be a large-egg fish; numbers of small-egg fish would remain unchanged above and below the Iskut because they were presumed to be migrating up the Stikine to Tahltan Lake.

The ratios of small-egg and large-egg fish were 1:0.21 below the Iskut and 1:0.15 above. This amounts to a 29% (1–0.15/0.21) reduction in the number of large-egg fish above the Iskut River, suggesting that some sockeye had indeed entered the Iskut. However, the difference was not significant (chi square 2 × 2 contingency test, $P > 0.5$), so no correction factor was used.

TABLE 2. Border escapement of Stikine sockeye salmon, 1983.

Fishery component	Total sockeye	Composition (%) Tahltan stock	Composition (%) River stock
Canadian commercial fishery	15 870	42	58
Canadian food fishery	4 649	90[a]	10[a]
Tahltan Lake escapement	21 256	100	0
River stock escapement	19 000 – 29 000	0	100
Border escapement	60 774 – 70 774	45–53	47–55

[a]Assumed.

Egg size factor

Based on available data, large-egg fish are not Tahltan stock, but it cannot be said with certainty that small-egg fish spawn only in the Tahltan Lake system. If some small-egg fish spawn at other locations, the present calculations underestimate the number of river spawners because all small-egg fish were assigned to the Tahltan stock.

Sex ratio factor

The above calculations assume that each stock is 50% males and 50% females. Preliminary scale analyses of commercial catches show 60% females for Tahltan fish and 40% females for the river stock (G. Oliver, ADFG, pers. comm.). If these ratios are accurate, the estimated proportion of Tahltan and river stocks in the commercial fishery would be 33 and 67%, respectively, and the actual number of river spawners would be 28 000 to 58 000 sockeye. For these ratios to be accurate, commercial catches must be unbiased with respect to sex, which is unlikely. Factors that probably bias the sex ratio in commercial gillnet samples are morphological differences in size and shape between (1) males and females of the same stock and (2) males of different stocks (early and late) when they pass through the fishery.

Border escapement equals spawning escapements (Tahltan and river stocks) plus catches at the in-river fisheries. In 1983 the border escapement was approximately 61 000 to 71 000 sockeye salmon (Table 2). If the stock composition of the food fishery, located near the confluence of the Tahltan and Stikine rivers, is assumed to be 90% Tahltan and 10% river stocks, then the stock composition of the border escapement is calculated to be 45–53% Tahltan and 47–55% river stock (Table 2).

The total exploitation rate of the border escapement in 1983 was 22–26% by the Canadian commercial fishery and 7–8% by the Native food fishery for a total of 29–34%. The total in-river exploitation rate of the Tahltan stock (34%) was somewhat higher than that of the river stock (25–34%) in the combined fisheries.

Comparison with Other Escapement Estimates

In 1983 there was a reasonably close agreement in estimates of border escapement derived from egg size analyses (61 000 to 71 000 sockeye) and scale pattern analyses (75 633; Walls 1984). In 1984, Lynch and Eggers (1985) compared border escapements calculated by these two methods with the estimate obtained by their sonar counter located near the Alaska–Canada border. Estimates based on egg sizes (derived in two different ways) were much closer (within 1–11%) to the sonar estimate of 83 121 than were estimates based on scale patterns (within

25–27%), probably because sockeye with Tahltan-type scale patterns have recently been found in non-Tahltan areas, as previously mentioned.

Discussion

The need to identify individual stocks of fish has long been a goal of fisheries managers, and a variety of biological characteristics have been examined for this purpose (Ihssen et al. 1981). The observation that some stocks differ in egg size may provide another management technique, especially in relatively simple systems where stocks in a drainage are few and widely separated in spawning location or run timing. The principal advantages of using egg size measurements in the Stikine drainage are that the technique costs little, the data are easily obtained, and the data provide *on-site* information about stock composition of the catch. The potential utility of this method may extend beyond the Stikine River. Gilhousen (1980) reported that similar racial differences also exist among sockeye in the Fraser River, wherein the earlier migrating fish generally have less developed gonads when they enter the river. This difference was less pronounced among Fraser males than females, which also seemed to be the case in the Stikine although this was not pursued beyond preliminary analyses.

Analysis of stock escapements in the Stikine River identified several potential sources of error which would also apply equally to other methods for stock separation in this drainage because most methods have relied on proportions of stocks in the in-river commercial fishery. Samples from this fishery may be biased in terms of sampling time, location, or sex ratio, all of which would affect escapement estimates of the river stock. Nonetheless, analyses by both scale pattern and egg size methods indicate that a significant proportion of sockeye in the Stikine drainage spawn at locations other than Tahltan Lake. During 1979–84, the border escapement of sockeye in the Stikine averaged 74 000 fish (range 34 000 to 139 000) of which an average of 54% (range 38–69%) were of non-Tahltan origin (McCart and Walder 1982; Oliver 1983; McPherson 1983; Walls 1984; Lynch and Eggers 1985; this study).

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Patterns of Aggressive Attack in Juvenile Steelhead Trout (*Salmo gairdneri*)

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Abbott, J. C., and L. M. Dill. 1985. Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*). Can. J. Fish. Aquat. Sci. 42: 1702–1706.

Nipping is a potentially damaging aggressive behavior of juvenile steelhead trout (*Salmo gairdneri*). By analyzing videotaped interactions of pairs of fish we determined where nips were aimed and where they contacted the opponent's body. Patterns of fin damage were also assessed. Aims and nips (contacts) were distributed differently in reciprocal and nonreciprocal aggressive bouts. In nonreciprocal bouts, aims were directed mainly at the dorsal fin, central body section, and caudal fin. Nip contact was biased towards the caudal fin. In reciprocal bouts, both aims and nips were concentrated on the dorsal fin and anterior portions of the body. We suggest that juvenile steelhead adjust their fighting tactics in response to opponent behavior. Mouth fighting, a novel behavior in juvenile trout, was observed in some reciprocal bouts. The dorsal fin incurred the greatest damage. We conclude that aggression (nipping) is responsible for the dorsal fin damage commonly observed in hatchery-reared salmonids.

Chez la truite arc-en-ciel (*Salmo gairdneri*) anadrome juvénile, le mordillage représente un comportement agressif potentiellement nuisible. En analysant les interactions de paires de poissons enregistrées sur bande vidéo, les auteurs ont déterminé les cibles et les points de contact corporel et ont évalué les caractéristiques des dommages aux nageoires. La répartition des cibles et des points de contact était différente entre les attaques agressives réciproques et non réciproques. Au cours de ces dernières, l'agresseur visait principalement la nageoire dorsale, le centre du corps et la nageoire caudale. Pour les attaques réciproques, les cibles et les points de contact étaient concentrés sur la nageoire dorsale et les parties antérieures du corps. Selon les auteurs, les juvéniles modifient leurs tactiques d'attaque en fonction du comportement de l'adversaire. Des combats bouche contre bouche, un nouveau comportement chez la truite juvénile, ont été observés au cours de certaines attaques réciproques où la nageoire dorsale a subi le plus de blessures. Les auteurs formulent la conclusion que l'agression (mordillage) est responsable des blessures aux nageoires dorsales fréquemment observées chez les salmonidés élevés en piscifacture.

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As aggressive animals functioning within either a dominance or territorial system, juvenile steelhead (*Salmo gairdneri*) require a mechanism by which contests are decided. Animals may settle disputes by direct, possibly damaging fighting (escalated fighting) or by more economical, ritualised forms of aggression (conventional fighting). Displays are used to compete for a resource or dominance position in a nondamaging manner, but these can escalate into direct physical combat (Maynard-Smith and Price 1973). Juvenile steelhead, in common with most other juvenile salmonids, employ visual displays of form, color, and movement. In escalated conflicts these displays are replaced by fighting in the form of nips (Chapman 1962).

Since Hoar's (1951) comparative work on juvenile *Oncorhynchus* spp., the behavior of several species of salmonids in both aquaria (Newman 1956; Keenleyside and Yamamoto 1962; Yamagishi et al. 1981) and streams (Kalleberg 1958; Chapman 1962; Jenkins 1969) has been observed and described. Behavior of *Salmo gairdneri* was first described by Stringer and Hoar (1955), and Hartman (1965) described the behavior of stream-dwelling juvenile steelhead. However, with the exception of work on reactive distance (Cole 1976; Dill 1978; McNicol and Noakes 1984), the form of salmonid agonistic behaviors has not been quantified. Nipping is potentially the most damaging behavior, and parameters of this behavior may be indicative of the costs of aggression, which if sufficiently high may reduce growth. Salmonids of hatchery origin commonly have damaged fins which may affect poststocking survival, increase the likelihood of disease, or make fish less acceptable to consumers. Such fin damage may be a consequence of aggression.

Our purpose was to measure the direction, site of contact, and result of nipping in juvenile steelhead trout and to compare patterns of aggression with observed patterns of fin damage.

Methods

Two groups of winter run Englishman River juvenile steelhead (24 and 53 d old, respectively) were obtained from the Fraser Valley Trout Hatchery, Abbotsford, B.C., in the spring and summer of 1981. The fish were starved for 24 h and then anesthetised, weighed, and measured (fork length). From each group, 10 lots of 8 similar sized fish were installed in 45-L flow-through aquaria. These remained between 8 and 11°C under 96 lx on a 14-h photoperiod. The fish were used in a

separate growth study and were videotaped under 2259 lx to record behavior, and weighed at the end of the 5-d experiment. Data from the two groups were pooled for analysis.

On the videotape, 100 random points were selected and the tape monitored until an aggressive bout occurred. A bout was characterized by the occurrence of a nip. If both fish delivered nips the bout was classified as reciprocal, and if only one of the pair delivered nips, nonreciprocal. Bouts were considered to have ended when the fish ceased to orient to one another.

Previous to administering a nip a fish would pause, orient towards the recipient, and then charge, generally in a straight line. Slow motion and stop action were used to isolate the frame in which the charge was initiated. A straight line was then passed through the eye and tail of the aggressor. The point where this line contacted the recipient was recorded according to the diagram in Fig. 1 (inset) and assumed to be the point at which the aggressor aimed it's nip. Slow motion and stop action were also used to isolate the point of contact in each bout. The body section on which a fish received a nip was recorded as with aim site. Charges that missed contact were included in the aim data set if part of a series of completed attacks. Eighty nonreciprocal bouts comprising 175 aims and 157 nips and 20 reciprocal bouts yielding 76 nips and 79 aims were viewed.

The resulting distributions of aims or nips were compared with a model distribution. For the model we assumed that any point on the fish had a random, or equal, chance of being scored. Thus, the relative area of each body section in Fig. 1 (inset) equalled the expected relative number of scores for that body section.

The two-dimensional nature of videotape analysis may have introduced error into the results. While measurement of attack contact area should be relatively unaffected, variation of position in the third dimension would affect the accuracy of measurement of aim site. However, there should not be any consistent directional bias. Aims that occurred when the attacker or the target was at an extreme angle towards or away from the plane of the screen were disregarded.

Fin damage of fish in videotaped groups and in 30 similarly treated groups of 8 was recorded at the conclusion of the experiments by examining them under a dissecting microscope. Only fish of groups with initially undamaged fins were used. If fins were split, or less than an estimated one third of a fin was missing, damage was classed as minor. When more than one third of a fin was estimated to be missing, damage was classed as severe. The distribution of fin damage was compared with a model distribution where each fin was assumed to have an equal chance of suffering damage. Scale loss and damage to the head (including jaws and gills) was scored for occurrence but not for severity.

Results

In reciprocal bouts both aims and nips were most frequently directed at the dorsal fin and anterior area of the body. The distributions of both aims and nips were significantly different from random (χ^2, $P < 0.001$) but were not significantly different from each other at the 0.05 level (Fig. 1). Nonreciprocal aims were most frequently directed at posterior areas of the body and the dorsal fin (Fig. 2). The distribution was significantly different from random (χ^2, $P < 0.001$) and also different from the distribution of aims in reciprocal bouts (χ^2, $P < 0.001$). Nips in nonreciprocal bouts were primarily recorded on the caudal fin and with decreasing frequency on the anterior regions. This

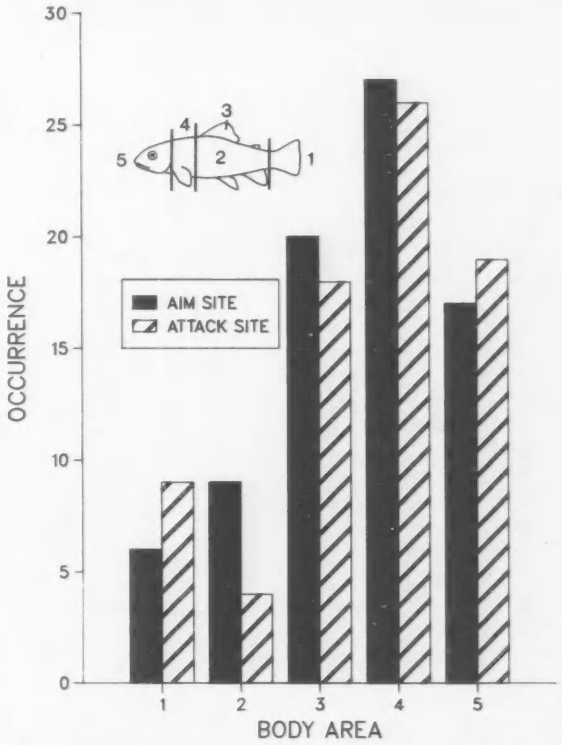

FIG. 1. Distribution of aims and nips in reciprocal aggressive bouts in juvenile steelhead trout.

pattern was significantly different from a random distribution, the pattern of nonreciprocal aims, and of reciprocal nips (χ^2, $P < 0.001$ in each case).

In all experiments the dorsal fin suffered the highest occurrence of minor damage. The pectoral and caudal fins were the second and third most damaged (Fig. 3). The occurrence of severe damage followed a similar pattern. No cases of damage to the anal fin were recorded. In all cases the distribution was significantly different from random (χ^2, $P < 0.001$).

In the course of videotaping groups, fish were observed to exhibit a previously undescribed behavior. On three occasions, two of which were on camera, fish ended a prolonged, reciprocal conflict by grasping each other's jaws and then "wrestling" by pulling, pushing, and twisting. This was termed mouth fighting (Fig. 4). These interactions occurred between vigorous similar-sized fish that appeared to have been contesting dominance of the tank. Mouth fighting occurred after intense bouts of fighting involving 37 and 77 nips/combatant (videotaped bouts). These nips were directed mainly at the head and pectoral fins as the fish circled. In two instances, pairs ceased nipping while facing one another and then closed and locked jaws. This was done at a lower speed than used when charging. In the third case the mouth locking occurred quickly in the course of a series of mouth-to-mouth nips. Fish remained locked for 27, 53, and 63 s. After releasing, one fish would swim away snapping its jaws and shaking its head, and then remain immobile near the surface of the tank with other subordinates. In the course of viewing videotapes of a separate study, three more instances of mouth fighting were observed; all followed a similar pattern.

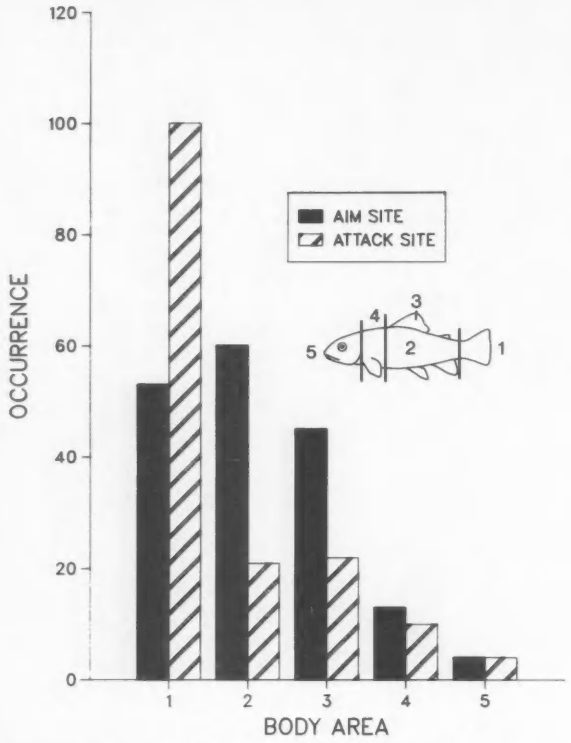

FIG. 2. Distribution of aims and nips in nonreciprocal aggressive bouts in juvenile steelhead trout.

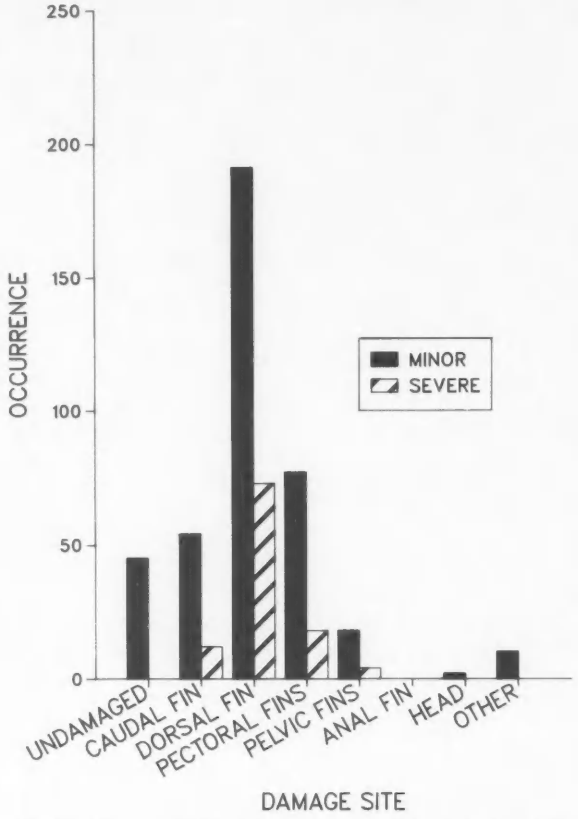

FIG. 3. Occurrence of physical damage in juvenile steelhead. Minor damage to fins is an estimated loss of <1/3 fin. Severe damage is an estimated loss of >1/3 fin.

Discussion

In reciprocal aggressive bouts juvenile steelhead aim and nip sites corresponded closely and were concentrated on the dorsal fin and anterior body regions. Nonreciprocal bouts also showed a pattern of aims directed at the dorsal fin, but nips were primarily on the posterior of the body.

The differences observed may simply be due to the retreat of one fish in the nonreciprocal bouts. Swimming away from the attack would cause the nip to contact behind the aim site. Alternatively, juvenile steelhead may employ different fighting tactics depending on the response of the opponent. The different reciprocal and nonreciprocal aim sites suggest such a change in tactics, since aim site should be relatively unaffected by target movement. Recipients rarely moved until the attack was initiated. We do not know whether head or tail nips are more damaging, but because nips to the head may cause damage to the gills, eyes, and mouth parts, such nips may be more dangerous and thus represent escalation. Fish were often observed to shake the head after receiving a nip there.

Literature reports of juvenile salmonid fighting behavior generally describe nips as being directed at the caudal area, e.g. by juvenile coho salmon, *Oncorhynchus kisutch* (Chapman 1962), and by Atlantic salmon, *Salmo salar* (Keenleyside and Yamamoto 1962). Stringer and Hoar (1955) stated that nips usually occurred close to the base of the tail in rainbow trout but acknowledged that this may be due to the rapid retreat of the recipient. Gibson (1981) observed biting at the flanks and caudal peduncle in four species of juvenile salmonids. Newman (1956) reported that fighting rainbow trout and brook charr (*Salvelinus fontinalis*) attempt to grasp the pectoral or dorsal fin of the opponent. These fish were held at high density and return nips were common, a situation corresponding to the reciprocal bouts of this study.

Orientation towards and damage to the dorsal fin may be expected if the dorsal fin is more conspicuous, more accessible, easier to grasp, or more delicate than other fins. The juvenile steelhead dorsal fin is usually spotted, and has a reddish hue and a white border at the tip, making it conspicuous. As such it may function in conflict as an aggressive releaser. Many juvenile salmonids show an erect dorsal fin in aggressive displays and a depressed fin in submissive postures (Newman 1956; Keenleyside and Yamamoto 1962; Chapman 1962; Jenkins 1969).

The complete absence of damage to the anal fin may indicate that it has little signal function or is for some reason less susceptible to attack. Dominant fish tended to hold a position in the center of the tank at mid-depth or higher. Thus most attacks were oriented downwards. Underwater observation of pen-held juvenile steelhead showed the most common attack to be a charge of two to three body lengths to nip the dorsal fin of deeper fish (personal observation by J.C.A). If this is the common attack behavior it would make contact with the anal fin unlikely.

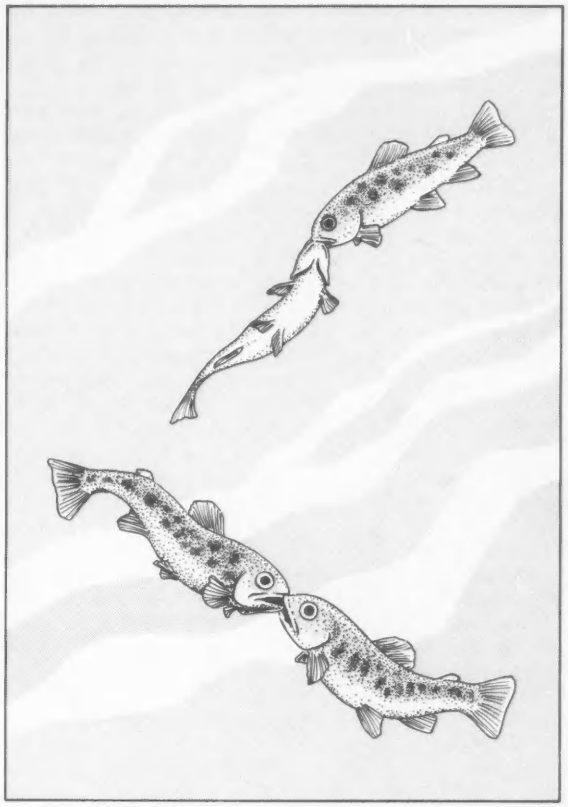

FIG. 4. Mouth fighting in juvenile steelhead. Outlines traced from videotape.

Several reciprocal bouts featured a previously undescribed behavior of juvenile steelhead. Mouth locking, although a common aggressive and courtship behavior in centrarchids and cichlids (e.g. Greenberg 1947; Baerends and Baerends-van Roon 1950), is not common in salmonids. MacPhee (1961) reported that both juvenile coho salmon and largemouth bass (*Micropterus salmoides*) locked jaws while dominance was first being established, presumably when intensity of aggression was high. R. Konopacky (Idaho Cooperative Fishery Unit, University of Idaho, Moscow, ID, pers. comm.) observed mouth-to-mouth contact of juvenile chinook salmon (*Oncorhynchus tshawytscha*) as territories were being established in a circular stream channel. Well-conditioned, dominant steelhead in this study were observed to have red lower jaws which may have a display function. It is not known if mouth fighting is relatively damaging to the combatants, but it appeared to be effective in ending the bout.

Fin damage in laboratory aquaria was concentrated on the dorsal fins, as would be expected from the observed attack behavior. This damage was similar to that observed in floating pen rearing facilities (personal observation by J.C.A.). Fin damage has been reported as a problem frequently affecting the culture of salmonids intended for commercial sale or for stocking in a sport fishery (Novotny 1980; Moring 1982). Dorsal fin height has even been used to distinguish wild from hatchery steelhead (Washington State Department of Game 1984 sport fishing regulations). Fin-damaged fish have a low angler acceptance (Whitlock 1974), probably display lower growth if stressed or if energy is being expended on repair of damaged tissue, and have greater susceptibility to bacterial fin disease (Schneider and Nicholson 1980). Damaged dorsal fins on lake pen-held fish were visible as white lines on their backs, while fish with little fin damage were relatively hard to detect. This may lead to differential predation following stocking. In addition the loss of fins may result in decreased maneuverability.

The fish culture literature has generally attributed reduced fins to erosion or "rubbing" (Schneider and Nicholson 1980; Gibson 1981; Moring 1982). While Boydstun and Hopelain (1977) observed startled steelhead rubbing their sides on net pens, it is hard to see how dorsal fins would commonly contact abrasive surfaces. Given the correspondence between attack sites and observed fin damage in aquaria and floating pens in this study, aggressive interaction may be the major cause of fin damage in hatchery salmonids.

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Partitioning Light Attenuation in an Acidic Lake[1]

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Effler, S. W., G. C. Schafran, and C. T. Driscoll. 1985. Partitioning light attenuation in an acidic lake. Can. J. Fish. Aquat. Sci. 42: 1707–1711.

Although a number of researchers have reported that acidification of lakes is accompanied by an increase in transparency, there has been no systematic evaluation of the processes responsible for this transformation. In this study we partitioned the attenuation of light in acidic Dart's Lake, located in the Adirondack region of New York from May to September 1982. We observed that changes in light attenuation (K_d) and light absorption (a) were regulated largely by "gelbstoff." Substantial decreases in K_d and a occurred through the study period and were correlated with a depletion in the concentration of dissolved organic carbon (DOC). In-lake concentrations of DOC were controlled by terrigeneous loading and in-lake processes. The decrease in DOC and the attendant decreases in a and K_d were coupled to a loss of Al from the water column of the lake. We suggest that coagulation/adsorption of DOC by Al may have contributed to increases in lake clarity. Increased transparency is significant because it enhances hypolimnetic heating and decreases the thermal stability of lakes.

Quoiqu'un certain nombre de chercheurs aient signalé que l'acidification des lacs est accompagnée d'une transparence accrue, aucune évaluation systématique des processus responsables de cette transformation n'a été réalisée. De mai à septembre 1982, les auteurs ont divisé l'affaiblissement de la lumière dans les eaux acides du lac Dart, situé dans les Adirondacks (État de New York). Ils ont observé que les variations de l'affaiblissement (K_d) et de l'absorption de la lumière (a) étaient principalement contrôlées par le « gelbstoff ». Des baisses importantes de K_d et de a ont eu lieu au cours de l'étude et correspondaient à un épuisement de la concentration de carbone organique dissous (COD). Les concentrations lacustres de COD étaient contrôlées par la charge terrigène et les processus du milieu. Une baisse du COD et les baisses correspondantes de a et de K_d correspondaient à une perte de Al dans la colonne d'eau. Les auteurs formulent l'hypothèse que la coagulation et l'absorption du COD par le Al peuvent avoir contribué à l'accroissement de la clarté des eaux. Une transparence accrue est importante car elle entraîne une augmentation du réchauffement de l'hypolimnion et une baisse de la stabilité thermique des lacs.

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The attenuation of diffuse light with depth in water, described by the attenuation coefficient for diffuse light (K_d, m^{-1}), is caused by absorption and scattering processes. A number of components in water contribute to the attenuation of light, including water itself, dissolved yellow substances ("gelbstoff"; adopted in this manuscript to reflect the optical properties of this heterogeneous group of materials), phytoplankton, and suspended inanimate particles. Water and gelbstoff absorb light, phytoplankton absorb and scatter light, and inanimate particles mostly scatter light. An evaluation of the contribution of these components to the attenuation of light has obvious value for lake systems in which the underwater light field has been altered as a result of anthropogenic activities. The interplay between phytoplankton biomass and attenuation has received considerable attention (e.g. Bannister 1974; Field and Effler 1983; Megard et al. 1979; Tilzer 1983). A manifestation of the eutrophication process is increased K_d (and decreased transparency). It is frequently assumed that the concentration of phytoplankton biomass regulates K_d, regardless of the level of productivity. However, examples of the regulation of K_d by components other than phytoplankton biomass have been reported. Kirk (1976) found that gelbstoff was the dominant component attenuating light in most of the highly colored (nonacidic) inland waters of Australia. Weidemann et al. (1985) demonstrated that the concentration of calcite particles was the most important factor regulating light attenuation for much of the summer period within mesotrophic Otisco Lake, NY.

The impact of the acidification process on attenuation apparently strongly contrasts that of eutrophication, as increases in transparency have been reported for a number of lakes following acidification (Almer et al. 1974; Malley et al. 1982; Schofield 1972; Yan 1983). Reductions in the attenuation of light may have profound effects on lake ecosystems as K_d strongly influences the attendant stratification regime. Increased clarity will encourage deeper epilimnia, reduced thermal gradients in the metalimnion (and therefore greater vertical mixing; Effler

[1]Contribution No. 33 of the Upstate Freshwater Institute, Inc.

and Field 1983), and increased hypolimnetic heating (Effler and Field 1983). Yan (1983) documented these effects that accompanied reacidification of Lohi Lake, Ontario. Certain shallow lakes, which routinely stratified prior to acidification, may fail to stratify as a result of acidification-induced decreases in K_d. The process(es) responsible for the reduction of K_d that accompany acidification has not been documented. Kramer (1978) speculated that the increase in Secchi disc transparency following acidification was "due to the dissolution of iron and manganese colloids and the decrease in organic detritus with decreasing pH due to decreasing photosynthesis." Based on a review of the literature, Yan (1983), however, concluded that the increase in transparency was not caused by reductions in phytoplankton biomass. Almer et al. (1978) hypothesized that increased transparency was due to decreases in "yellow organic acids" (gelbstoff), caused by precipitation with Al at low pH. Unfortunately, there has never been a systematic evaluation of the processes and components regulating K_d in acidic lakes. Partitioning light attenuation in acidic systems according to the contributions of the processes of absorption and scattering, and according to selected components, would aid in identifying the mechanisms responsible for changes in the clarity of acidic lakes.

In this study we tested the hypothesis of Almer et al. (1978) that transformations in light attenuation in acidic lakes are regulated by changes in gelbstoff. In addition, we considered the role of Al in depleting dissolved organic carbon in the water column and the associated increases in transparency.

Description of Dart's Lake

The study site, Dart's Lake, is located in the Adirondack Mountain region of New York State (72°52′W, 43°48′N). The watershed, which occupies 107 km^2, is forested with minor areas of exposed bedrock. The lake volume is 4.1×10^6 m^3 with a mean depth of 7.1 m and a maximum depth of 16 m. Dart's Lake has a single outlet, and our water budget indicates that 95% of the outlet flow (7.25×10^7 $m^3 \cdot yr^{-1}$) enters the lake at the major inlet. A detailed description of the water chemistry of Dart's Lake has been presented elsewhere (Driscoll and Schafran 1984a, 1984b).

Methods

Downwelling (E_{od}') and upwelling (E_{ou}') irradiances were measured with a Protomatic underwater light meter that was equipped with two spherical sensors (exposed as hemispheres). The conformations of the upward and downward sensors were appropriate for measuring quantities proportional to the sum of scalar (E_o) and vector (E) irradiance and the difference between E_o and E, respectively (Hojerslev 1975). The measurements were made at 0.5-m depth intervals at a central deepwater (15 m) location on seven dates from mid-May to early October 1982 between 11:30 and 13:00. Reflection from the lake bottom did not contribute significantly to the upwelling measurements. The vector irradiance (E) was determined as the difference between upwelling and downwelling measurements ($E = E_{od}' - E_{ou}'$); E_o was determined as the sum of these measurements ($E_o = E_{od}' + E_{ou}'$). The wavelength response of this meter is similar to the ideal quantum response for PAR (Field and Effler 1983).

The attenuation coefficient (K_d, m^{-1}), an apparent optical property (Kirk 1981a, 1981b), and the absorption coefficient

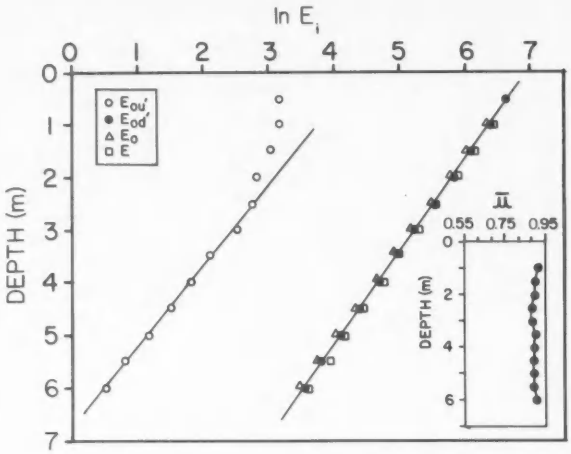

FIG. 1. Typical irradiance profiles for Dart's Lake, 25 June 1982. Inset: typical profile of $\bar{\mu}$, for the same day.

(a, m^{-1}), an inherent optical property (i.e. its value at a given point in a given medium is independent of changes in the radiance distribution; Preisendorfer 1961), were determined from field measurements. The attenuation coefficient K_d was determined according to the Lambert–Bouguer Law; operationally, K_d was calculated as the slope of the linear regression analysis of ln (E_{od}') on depth (Z). The absorption coefficient a was determined according to the following expression (Hojerslev 1975):

$$(1) \quad a = K_E \cdot E/E_o$$

where K_E is the vector irradiance attenuation coefficient (m^{-1}). The value of K_E was essentially equivalent to K_d in Dart's Lake (e.g. Fig. 1). The ratio E/E_o is equivalent to the average cosine ($\bar{\mu}$; the average cosine of the angles of the photon trajectories to the vertical). Optical characterization of the water column (K_d, $\bar{\mu}$, and a) was limited to the epilimnion to avoid the discontinuities associated with stratification. Greater than 95% of the incident light was attenuated within the epilimnion on all the monitoring days. The standard curves of Kirk (1981b) were used to estimate the scattering coefficient b at the 10% light level (middle of the photic zone) from $\bar{\mu}$. The values of a and b, determined from these methods, are averages for the waveband detected. This effect is minor for b, as it does not vary markedly with wavelength.

The uncertainty in the determination of K_d was represented as the 95% confidence interval of the slope of the regression line. Uncertainty in $\bar{\mu}$ (E/E_o) was represented by ±2 standard deviations about the mean. The uncertainty in the estimate of a accommodated the uncertainties in both $K_E(K_d)$ and $\bar{\mu}$ (as per Eq. (1)).

Results

Semilogarithmic plots of irradiance as a function of depth are shown in Fig. 1 for E_{od}', E_o, E_{ou}', and E for 25 June 1982. The closeness of E_{od}', E_o, and E is characteristic of a relatively small contribution to attenuation by scattering. The attendant depth distribution of $\bar{\mu}$ for the same day is presented as an inset in Fig. 1. The linearity of the irradiance plots, and the uniform depth distribution of $\bar{\mu}$, observed on this date were typical of the

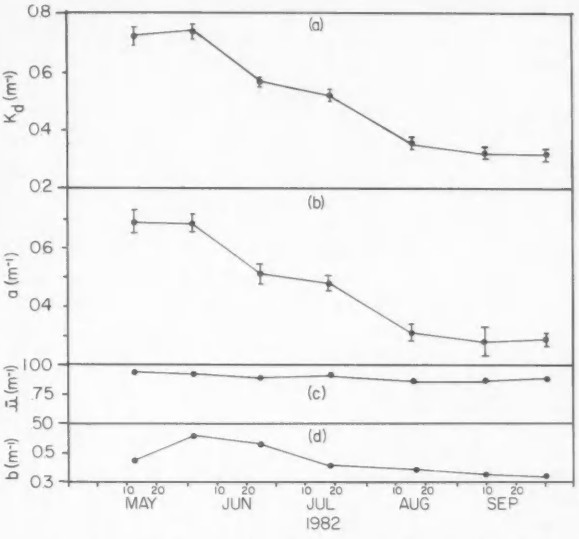

FIG. 2. Temporal variations in optical properties (a) K_d, (b) a, (c) $\bar{\mu}$, and (d) b in Dart's Lake for the study period, 1982.

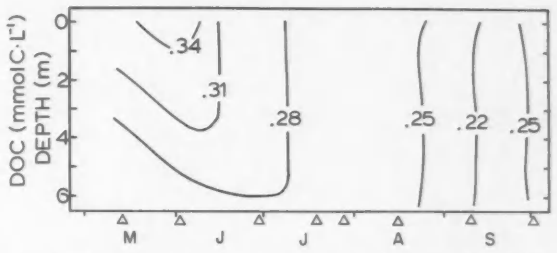

FIG. 3. Isopleth of DOC in the epilimnion of Dart's Lake, 1982.

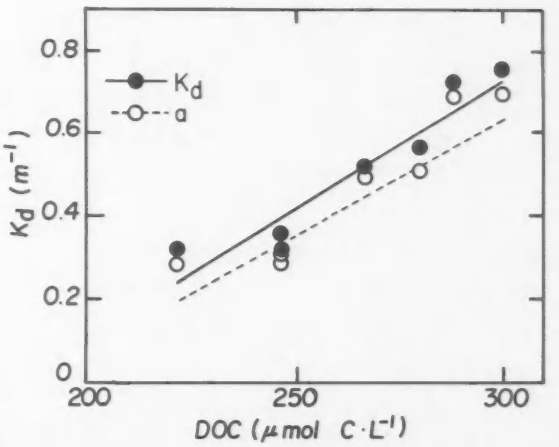

FIG. 4. K_d and a as a function epilimnetic DOC for Dart's Lake, 1982.

conditions monitored on the other 6 d. Substantial temporal variations in K_d and a occurred during the study period (e.g. compared with the level of uncertainty in the measurements; Fig. 2a and 2b). The temporal distributions of K_d and a were highly correlated ($r^2 = 0.999$); thus, $\bar{\mu}$ remained relatively uniform by comparison (Fig. 2c), although a minor decrease occurred through mid-August. The value of b was low throughout the study; it decreased progressively from early June. However, substantial uncertainty (approximately ±25%) accompanied these estimates of b because of the great sensitivity of the b/a ratio to zenith angle for the high values of $\bar{\mu}$ (Kirk 1981b) observed in Dart's Lake.

Discussion

Gelbstoff and Attenuation

The transformations in K_d observed in Dart's Lake were clearly regulated by changes in a. Only the slight increase in K_d from mid-May to early June (not significant at the 95% confidence level) may have been caused by a change (increase) in b. The nature of the transformations and the relative magnitudes of a and b lead us to conclude that gelbstoff was the component regulating the transformations in a, and therefore K_d. The high values of $\bar{\mu}$ observed for Dart's Lake are common to humic (gelbstoff) lakes that are low in suspended material (Howard-Williams and Vincent 1984). Phytoplankton biomass apparently was insignificant in the attenuation of light. This observation is based on the low b/a ratio of the lake (0.65–1.25), which was much smaller than the magnitude associated with phytoplankton (Bricaud et al. 1983), and the very low attendant concentration of chlorophyll a ($\leq 0.3\ mg \cdot m^{-3}$; Driscoll and Schafran 1984a). By utilizing a reasonable value (Bannister and Weidemann 1984) for the upper limit of the mean spectral chlorophyll a specific absorption coefficient, we estimate that the phytoplankton component of a was less than $0.003 \cdot m^{-1}$ throughout the study period. The spectral average value of the absorption due to water is somewhat dependent on the wavelength distribution (Smith and Baker 1981), but its magnitude is substantially less than the values of a determined here.

More direct support for the position that gelbstoff regulated the observed transformations in K_d and a in Dart's Lake is provided by the analysis of attendant dissolved organic carbon (DOC) data (Fig. 3). In the absence of gelbstoff (optical) measurements (e.g. Bricaud et al. 1981; Kirk 1976) we have adopted DOC as a surrogate measure, as this chemical analysis is expected to include most of the light-absorbing dissolved colored substances. We acknowledge that DOC is an imperfect surrogate of gelbstoff (e.g. light-absorbing characteristics of organics are somewhat compound specific). The concentration of DOC in the epilimnion of Dart's Lake remained relatively constant through June and decreased progressively until mid-September (Fig. 3). Temporal transformations in DOC explained much of the observed variation in K_d ($r^2 = 0.90$, $p < 0.01$) and a ($r^2 = 0.92$, $p < 0.01$; Fig. 4). The linear regression analyses should not be extended beyond the limits of the observations. This limitation is evident by the negative intercepts obtained by the regression analyses ($K_d = -1.2 \cdot m^{-1}$ and $a = -1.1 \cdot m^{-1}$ for DOC = 0.0 μmol $C \cdot L^{-1}$), indicating the apparent nonlinearity of the relationships for DOC concentrations less than those observed in this study. This nonlinearity is to be expected, as the absorption by water would become relatively more important at lower concentrations of DOC. We consider the results of Fig. 4 strong support for the importance of DOC (i.e. gelbstoff) in regulating transformations of K_d and a in Dart's Lake.

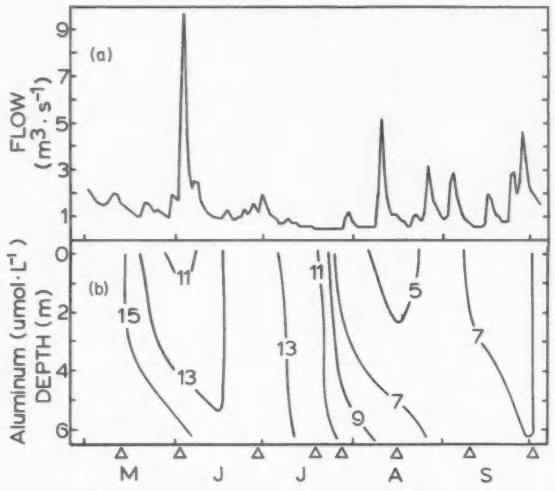

FIG. 5. (a) Hydrograph for the inlet of Dart's Lake, 1982; (b) isopleth of Al in the epilimnion of Dart's Lake, 1982.

Loading and In-Lake Processes

An evaluation of the loading of materials to the lake provides additional information on the variations in light attenuation observed in Dart's Lake. The hydrograph for the single major inlet to Dart's Lake from the beginning of May through the monitoring period is shown in Fig. 5a. Unfortunately, because concentrations of suspended solids were extremely low (<1 mg·L^{-1}), they were measured at infrequent intervals. Although values were generally low, the concentration of particulate matter entering the lake undoubtedly increased during runoff events. The peak in *b* (Fig. 2d) in early June coincided with a major rainfall event (Fig. 5a). However, terrigeneous inputs of particulate matter alone may not explain the estimated variations in *b* in Dart's Lake, as smaller hydrologic events in late August and September did not result in increases in *b*.

The major source of gelbstoff to Dart's Lake appeared to be stream inputs. Autochthonous generation of DOC associated with phytoplankton productivity (Sharp 1977; Bricaud et al. 1981) was undoubtedly low in view of the extremely low standing crop of phytoplankton. The trend in DOC influx to Dart's Lake was similar to the hydrologic loading, as the concentration of DOC in the inlet stream was relatively constant (225 ± 8 μmol C·L^{-1}, with the exception of a peak value of 425 μmol C·L^{-1} during the June rainfall event). The high DOC concentrations in the epilimnion of Dart's Lake following snowmelt, the increase in DOC associated with the early June runoff event, and the minor increase in early October reflect seasonal variations in water column gelbstoff and the effect that terrigenous inputs may have on the attenuation of light (Fig. 5a).

The seasonal variation in light attenuation appeared to be mediated by variations in the DOC (gelbstoff) concentration in Dart's Lake. The progressive depletion of DOC and decrease in K_d through the summer season may have been facilitated by in-lake removal of Al. Driscoll and Schafran (1984b) reported that depletion of NO_3^- resulted in the generation of acid neutralizing capacity and the removal of Al from the water column of Dart's Lake during summer stratification. The depletion of DOC coincided with the removal of Al from the photic zone of the lake (Fig. 5b; DOC = 168 + 10.6 Al where DOC and Al are in micromoles per litre, $n = 29$, $r^2 = 0.45$, $p < 0.0001$). Other investigators have also reported that the transformation of Al from aqueous to particulate phases facilitates the removal of DOC from acidic lakes (Dickson 1978; Driscoll et al. 1982). Our observations for Dart's Lake are consistent with the hypotheses of Almer et al. (1978): (1) transformations in light attenuation were regulated by changes in the concentration of DOC and (2) these changes in the concentrations of DOC may have been facilitated by coagulation with Al.

The ubiquity of the attenuation characteristics and the regulatory mechanisms described here for Dart's Lake are, of course, not established. However, Driscoll and Newton (1985) observed elevated concentrations of DOC in a study of 18 Adirondack drainage lakes. Unpublished data by C. T. Driscoll indicate that concentrations of chlorophyll *a* in these lakes are comparable with or below values in Dart's Lake. In addition, it has been reported (C. T. Driscoll, C. P. Yatsko, and F. J. Unangst, in review), for the same data set, that DOC and Al concentrations were highest in Adirondack drainage lakes following spring snowmelt and decreased during the summer season. Comparable water quality characteristics suggest that the optical phenomena observed for Dart's Lake may occur widely. However, a more complete analysis, with additional optical measurements (e.g. Howard-Williams and Vincent 1984; Kirk 1981b; Weidemann et al. 1985), of more lakes in acid-impacted regions is essential to evaluate the applicability of our observations for Dart's Lake. This recommendation is particularly important in light of the critical role K_d plays in the stratification regime, and the apparent impact acidification has had on K_d.

Acknowledgments

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Stable Carbon and Nitrogen Isotope Tracers of Trophic Dynamics in Natural Populations and Fisheries of the Lahontan Lake System, Nevada

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Estep, M. L. F., and S. Vigg. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake system, Nevada. Can. J. Fish. Aquat. Sci. 42: 1712–1719.

The influence of combined inorganic N on the growth of N_2-fixing blue-green algae (*Aphanizomenon flos-aquae*) in Lahontan Reservoir was detected with N isotopic compositions ($\delta^{15}N$ = +1.2 to +7.0) that were enriched in ^{15}N. In Pyramid Lake, however, the $\delta^{15}N$ of the entire food web was influenced by a contribution of isotopically light N released from N_2-fixing blue-green algae (*Nodularia spumigena*). Carbon isotope ratios ($\delta^{13}C$) of these unpalatable, blue-green algae from both lakes were different enough from the zooplankton and higher animals to preclude a direct trophic link. An enrichment in ^{13}C with trophic level in the food chain was measured in both lakes. Carbon isotope ratio measurements clearly illustrated the isotopic similarity between hatchery-raised Lahontan cutthroat trout (*Salmo clarki henshawi*) and cui-ui (*Chasmistes cujus*) and the artificial diet, but differed from the $\delta^{13}C$ of their wild counterparts consuming natural foods. There is a consistent isotope fractionation between the $\delta^{13}C$ of scales and the $\delta^{13}C$ of muscle from fish that is species specific. This finding demonstrates that fish scales, as well as muscle, can be used to determine diet.

L'influence de l'azote inorganique combiné sur la croissance d'une algue bleu-vert (*Aphanizomenon flos-aquae*) fixatrice de l'azote dans le réservoir Lahontan a été détectée avec des compositions isotopiques d'azote ($\delta^{15}N$ = +1,2 à +7,0) enrichies de ^{15}N. Dans le lac Pyramid, le $\delta^{15}N$ de toute la chaîne alimentaire était toutefois sous l'influence d'un apport d'un isotope léger de N émis par une algue bleu-vert (*Nodularia spumigena*) fixatrice de l'azote. Les rapports des isotopes de C ($\delta^{13}C$) dans ces algues bleu-vert désagréables au goût provenant de ces deux lacs étaient suffisamment différents de ceux du zooplancton et des animaux supérieurs pour exclure un lien trophique direct. On a quantifié l'enrichissement en ^{13}C en fonction du niveau trophique de la chaîne alimentaire dans les deux bassins. Les quantifications du rapport des isotopes de C ont clairement illustré la ressemblance isotopique entre, d'une part, la truite fardée de Lahontan (*Salmo clarki henshawi*) et le cui-ui (*Chamistes cujus*) produits en écloserie et, d'autre part, les aliments artificiels; ces quantifications étaient toutefois différentes de $\delta^{13}C$ présent chez leurs cousins sauvages se nourrissant d'aliments naturels. Il existe un fractionnement isotopique uniforme particulier à chaque espèce entre le $\delta^{13}C$ des écailles et le $\delta^{13}C$ des muscles de poisson. Cette découverte révèle que les écailles de poisson ainsi que les muscles peuvent être utilisés pour établir le régime alimentaire.

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Energy flow through trophic levels of biological systems can sometimes be traced by measuring variation in the natural abundance of stable isotopes (e.g. C, N, H, and S). Stable isotopes can be useful tracers because inorganic nutrients as well as organic dietary sources in the food web may have different isotopic compositions (see Fry and Sherr 1984 for a recent comprehensive review). In plants the isotopes in inorganic C and N sources are fractionated as the elements are metabolized by specific biochemical pathways. For example, C isotope ratios can distinguish between vascular plants that fix C from CO_2 into a 3- versus 4-carbon molecule (Smith and Epstein 1971). Stable N isotopic compositions may distinguish plants that fix atmospheric N_2 from those that metabolize soluble nitrogenous compounds (Virginia and Delwiche 1982).

In recent years, stable isotope techniques have been successfully applied to the study of bioenergetics in estuarine and marine ecosystems. Marine algae and phytoplankton commonly have C and N isotopic compositions different from those of terrestrial higher plants and seagrasses. The relative importance of each type of plant to the food web can thus be assessed.

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In contrast, stable isotopes have had only minimal application to freshwater ecology. Differences in the isotopic compositions of the primary producers, algae and phytoplankton, can be more variable in freshwater systems. For example, in several New England lakes, Oana and Deevey (1960) measured a 10‰ variation in the C isotope ratios of plankton, which was due to differences in the $\delta^{13}C$ of the inorganic C. The influence of seasonal variations in the biogeochemical cycle can also change the $\delta^{13}C$ of phytoplankton by 15‰ (Stiller and Nissenbaum 1980). In a food web study, Rau (1980) used C isotope ratios to determine the contribution of allochthonous organic matter to aquatic insects in a subalpine lake. Examples of N isotope studies in freshwater systems are fewer. Pang and Nriagu (1977) measured $\delta^{15}N$ of phytoplankton and zooplankton samples from Lake Superior in addition to their extensive study of N fractions from sediments. In comparison with marine plankton, the lacustrine samples had more negative $\delta^{15}N$ values. The behavior of multicomponent stable isotope traces of organic substances in lakes and rivers is not well defined or understood.

In this paper several potential applications of the stable isotope technique as a tracer of biological processes in limnology and freshwater fisheries have been explored in two contrasting lakes in northern Nevada: Pyramid Lake and Lahontan Reservoir. Firstly, the effects of pollution on the N cycle were measured on inorganic and organic N in Lahontan Reservoir. Allochthonous input of N into a lake limited with respect to this nutrient can be calculated by measuring flow rates of rivers emptying into the lake and the concurrent concentrations of inorganic N species on a temporal basis. The fate of this N and its residence time in the lake are not straightforward measurements, but are crucial to the ecology of the organisms and the biogeochemical cycling. Stable isotopes could be useful tracers of particular sources of N because the isotopic conpositions of N in either inorganic species or organic matter may be source and pathway dependent.

Secondly, the $\delta^{15}N$ of blue-green algae from Lahontan Reservoir were related to the number of heterocysts and their biochemical activity. Many filamentous blue-green algae that are capable of fixing N_2 from the atmosphere have specialized cells called heterocysts that contain the enzymes necessary for N_2 fixation. These blue-green algae are stimulated by low, combined-N levels to form heterocysts because the presence or uptake of ammonia often represses the induction of heterocyst formation (Bottomley et al. 1979). Other strains of blue-greens, however, continue to have active nitrogenase, the enzyme responsible for fixing N_2 (Bottomley et al. 1979), even when ammonia is still available. The $\delta^{15}N$ of N_2-fixing blue-green algae that have been measured is approximately 0.0‰ (Wada and Hattori 1976; Macko et al. 1982; Estep and Macko 1985) because there is little isotope fractionation during N_2 fixation (Hoering and Ford 1960; Macko et al. 1982).

Thirdly, one of the questions concerning N-limited lakes is the relative importance of N_2-fixing blue-green algae to the nutrient cycle. Presumably, these algae are unpalatable to most zooplankters, the primary diet of small fish (Cooper and Vigg 1985). Both lakes host a considerable population of filamentous blue-green algae during the summer months. The importance of these organisms to the N cycle and the food chain is assessed.

Fourthly, stable isotopes have been used as tracers of multicomponent diets through the food webs of the two lakes, which have distinctive fish populations. Pyramid Lake has a native fish fauna endemic to the Lahontan Basin (Vigg 1981). Notably, cui-ui (*Chasmistes cujus*), an endangered species found only in Pyramid Lake, and Lahontan cutthroat trout (*Salmo clarki henshawi*) are both threatened by lack of natural reproduction. Lahontan cutthroat trout achieves its potential as a lacustrine piscivore only in the uniquely suitable environment of Pyramid Lake. Lahontan Reservoir, in contrast, has an exotic and diverse fish fauna with little resemblance to the short and efficient food chain that evolved in Pyramid Lake. Introduced species, including carp (*Cyprinus carpio*), catfish (*Ictalurus* spp.), Sacramento blackfish (*Orthodon microlepidotus*), and white bass (*Morone chrysops*), dominate the fish community.

Lastly, fish hatcheries and rearing facilities are managed by the Pyramid Lake Paiute tribe, who stock both cui-ui and Lahontan cutthroat trout into the lake in large numbers. The studies presented here are attempts at defining the isotopic relationship of a hatchery fish with its food and comparing hatchery-reared animals with wild ones. In addition, the isotopic composition of fish scales is compared with that of muscle to evaluate the use of tracing diet and potentially tracing age-specific scale regions to trophic level. Obviously, owing to depleted population levels and their legal status as an endangered species, cui-ui cannot be sacrificed for food-habit studies. Thus, sampling of scales is a nondestructive method for accomplishing this objective.

Study Sites

The Truckee River, Pyramid Lake, and Lahontan Reservoir are remnants of late-Pleistocene Lake Lahontan that inundated some 22 300 km^2 during the last pluvial period, about 12 000 yr ago. The Truckee River emanates from oligotrophic Lake Tahoe in the Sierra Nevada; it flows about 192 km and descends 715 m before entering the southwest end of Pyramid Lake (Fig. 1). Snowpack is the primary source of the river; maximum flows occur during the spring snowmelt, and the historical mean annual discharge is about 5.9×10^8 m^3. Upstream, the river water has a nearly neutral pH and a low ionic concentration (<75 mg total dissolved solids (TDS)/L). It receives a large anthropogenic load of nutrients (especially N) from the Reno–Sparks Joint Water Pollution Control Plant (R-SJWPCP) at its midreach; and in the lower river, seasonal low flows, high pH (>8), high temperature (>20°C), and large periphyton biomass occur.

Pyramid is a terminal lake, i.e. it has no outflow. This lake is saline (~5 g TDS/L), is N limited, has seasonal blooms of the blue-green alga *Nodularia spumigena*, and is classified as mesotrophic (Galat et al. 1981).

Derby Dam, constructed in 1905, is situated on the Truckee River, 56 km upstream from Pyramid Lake. It diverts water via the Truckee Canal to Lahontan Dam and Reservoir, which were completed in 1915 on the lower Carson River. In addition to receiving the entire flow of the Carson, Lahontan Reservoir has historically received about half of the total flow of the Truckee River. The reservoir water is fresh (<200 mg TDS/L), is N limited, has seasonal blooms of the blue-green alga *Aphanizomenon flos-aquae*, and may be considered eutrophic (Cooper et al. 1983).

Methods

Water, sediment, and biological samples were collected from Pyramid Lake, Lahontan Reservoir, the Truckee River, the R-SJWPCP, and the Truckee Canal during 1982–84. Biological samples were kept frozen until they were dried at 60°C. Before

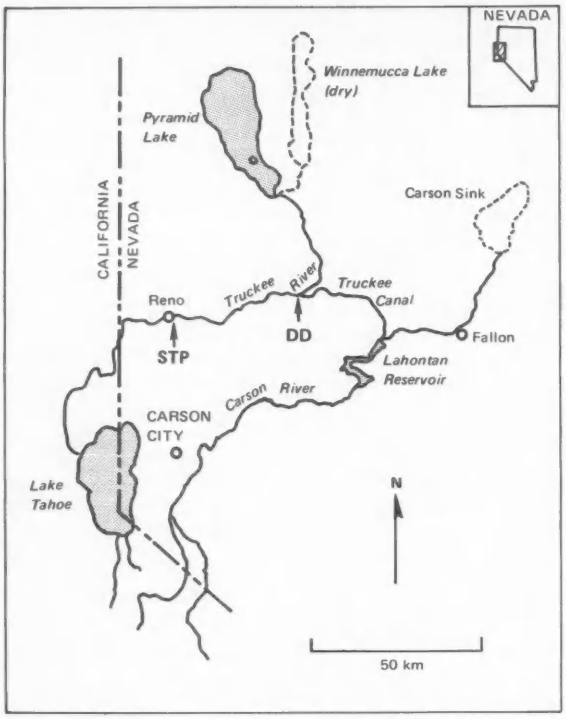

FIG. 1. Truckee River drainage basin including Pyramid Lake and Lahontan Reservoir, Nevada. DD = Derby Dam; STP = sewage treatment plant.

dissection, the thawed fish were washed with tap water. The muscle of fish was used routinely as the representative sample of the whole fish. Fish scales and sediment were acidified with 2 mol HCL/L, which was then decanted to remove carbonate and surface bacteria prior to analysis. The N isotopic composition of dissolved inorganic N species was determined on H_2O samples after centrifugation followed by filtration through a 0.8-μm Millipore filter to remove fine organic materials. The organic residue was dried in an oven at 60°C and the supernatant and filtrate were evaporated. These samples were subsequently preserved in H_2SO_4. The organic residue was subjected to Kjeldahl digestion, and the ammonia from either the water or organic samples was distilled quantitatively into a solution of HCl and subsequently dried before analysis. A known sample of ammonium chloride was subjected to the distillation process as a control. The $\delta^{15}N$ of the initial NH_4Cl control was +0.3‰, and after processing it was +0.2‰.

For the analysis of the stable C and N isotopic composition, samples were converted to CO_2 and N_2 by a modification of the Dumas dry-combustion method (Stump and Frazer 1973). The dried powder was mixed with high-purity copper and precombusted copper oxide in a quartz tube, which was then evacuated, sealed, and combusted at 900°C for 1 h. CO_2 and N_2 were isolated cryogenically and analyzed with isotope-ratio mass spectrometers (Nuclide Corp., model RMS-6-60, for CO_2, and a similar type of instrument constructed at the Geophysical Laboratory for N_2).

The stable isotopic ratio is calculated in terms of δX as follows:

$$\delta X = \left[\frac{R \text{ sample}}{R \text{ standard}} - 1\right] \times 10^3(‰)$$

where $X = {}^{13}C$ or ${}^{15}N$ and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. The standard for C is the Peedee Belemnite (limestone) that has been assigned a $\delta^{13}C$ value of 0.0. The standard for N is air N_2: $\delta^{15}N = 0.0$. Samples with positive isotope ratios are enriched in the heavy isotope relative to the standard, whereas those with negative isotope ratios are depleted with respect to the standard. The reproducibility of the measurement is ±0.1 for $\delta^{13}C$ and ±0.2 for $\delta^{15}N$.

Results and Discussion

Tracing the Inorganic Source of N with Stable Isotopes

Lahontan Reservoir usually receives about 50% of its total N from the Truckee Canal and the remainder from the Carson River, according to a simplified model which assumes that N_2 fixation is negligible (Cooper et al. 1983). In 1983, however, input from the Carson River was much greater due to high river flows. The total inorganic N in the Truckee River above the sewage-treatment plant has a $\delta^{15}N$ value (−1 to +1) typical of input from fertilizer and the natural biogeochemical cycle (Kreitler 1975; Table 1). Before the water enters the Truckee Canal, a substantial amount of inorganic N enters from the effluent of the R-SJWPCP that has an isotopic composition almost 9‰ heavier than that of the incoming, ambient N. Because the concentrations of N are at least 100 times background, the periphyton in the Lower River and Truckee Canal are receiving an elevated amount of N compared with the Upper Truckee River and Lahontan Reservoir. Algae growing in nonlimiting conditions will incorporate isotopically light N into their cellular proteins (Wada and Hattori 1978; Macko et al. 1982). The residual total inorganic N is thus enriched in ${}^{15}N$.

Within Lahontan Reservoir, where the summer, growing-season levels of dissolved N are essentially zero, the $\delta^{15}N$ of inorganic N is only +1.1, yet the $\delta^{15}N$ of algae (blue-green) is +8.4. Thus, the isotopic composition of N_2-fixing blue-green algae in Lake Lahontan is anomalous and indicates an additional N source, perhaps the isotopically heavy N from the polluted Truckee Canal or from unmeasured sources along the Carson River.

Relationship of N_2 fixation to the N isotopic composition of blue-green algae

The number of heterocysts and their biochemical activity are related to the $\delta^{15}N$ of *A. flos-aquae* in the same samples (Table 2). In Lahontan Reservoir, samples with an increased number of heterocysts (>20 per 1000 cells) have $\delta^{15}N$ nearer to the expected value of 0.0‰. Samples with <20 heterocysts per 1000 cells have $\delta^{15}N$ considerably enriched in ${}^{15}N$. A more complex N metabolism for these algae is indicated by the stable N isotopic compositions, which are more sensitive indicators than biochemical measurements of the relative importance of N_2 fixation and inorganic nutrient uptake. The blue-green algae in Lahontan Reservoir are not only fixing N, but are incorporating combined inorganic N from some other source.

Tracing Food Webs with C and N Isotopes

Pyramid Lake studies

Algae and zooplankton from Pyramid Lake have $\delta^{13}C$ values that are distinctly different from those of blue-green algae

TABLE 1. Concentration and N isotopic composition of inorganic and organic nitrogenous compounds from the Truckee–Lahontan Reservoir water system, October 1982 (nd = not detected).

Location and sample	NO_2^- ($mg \cdot L^{-1}$)	NO_3^- ($mg \cdot L^{-1}$)	NH_4^+ ($mg \cdot L^{-1}$)	Dissolved organic N ($mg \cdot L^{-1}$)	$\delta^{15}N$ (‰)
Upper Truckee River					
Inorganic N	<0.03	0.09	<0.06	nd	+0.4
Reno–Sparks sewage plant					
Inorganic N	nd	0.49	15.67	0.76	+9.0
Organic sewage effluent					+1.0
Truckee Canal					
Inorganic N	0.01	0.21	<0.05	nd	+17.6
Algal residue					+7.0
Lahontan Reservoir					
Inorganic N	<0.01	<0.05	<0.05	nd	+1.1
Algae Aug. 1981					+8.6
Algae Oct. 1982					+8.2

TABLE 2. Relationship of N_2 fixation to the stable isotopic composition of blue-green algae[a] from Lahontan Reservoir, July–September 1983 (nd = not determined).

Sample date 1983	Algal cells (mL)	Heterocysts per thousand cells	Heterocysts per mL	N_2 fixation (nmol $C_2H_4 \cdot L^{-1} \cdot h^{-1}$)[b]	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
6 July	7 161	17.3	124.2	nd	+3.2	−32.0
13 July	2 973	21.0	62.5	nd	+1.2	−29.1
2 Aug.	4 730	11.6	54.7	nd	+5.2	−30.0
24 Aug.	12 347	3.6	44.8	37.1	+5.2	−30.8
7 Sept.	32 632	5.7	186.3	31.1	+5.8	−28.9
13 Sept.	1 546	4.1	6.3	3.8	+4.4	−26.9
20 Sept.	38 999	1.3	50.7	221.5	+4.1[c]	−30.2[c]
					+6.5[d]	−29.5[d]
22 Sept.[e]	9 737	1.6	15.4	5.7	+7.0	−28.9

[a]Primarily the species *Aphanizomenon flos-aquae*.
[b]Determined by acetylene reduction.
[c]Offshore sample.
[d]Inshore sample.
[e]Algae collected from the beach.

TABLE 3. Isotopic composition of algae and zooplankton from Pyramid Lake, 1983–84.

Sample No.	Taxon	$\delta^{15}N$ (‰)	$\delta^{13}C$ (‰)
Δ22	Blue-green algae (*Nodularia*)	+2.9	−17.3
Δ14	Green algae (*Cladophora*) A	+6.0	−23.3
Δ15	B	+4.2	−23.3
Δ12	Chironomids (on green alga B)	+6.5	−23.0
Δ17	Zooplankton May 1983	+9.2	−24.0
Δ48	Zooplankton Feb. 1984	+11.2	−24.5

(Table 3). Zooplankton probably feed primarily on periphyton and phytoplankton (primarily diatoms), and not planktonic blue-green algae (Galat et al. 1981). Although no actual diatomaceous phytoplankton sample was measured from Pyramid Lake, the value for $\delta^{13}C$ could be predicted to be about −25‰ from previous studies that have defined the relationship between $\delta^{13}C$ of an animal and its diet (Gearing et al. 1984). For N isotope ratios, progressive enrichments of +2 to +3‰ with each trophic level have been measured in marine and laboratory environments (Miyake and Wada 1967; Minagawa and Wada 1984). The zooplankton were not obviously consuming blue-green algae, as the difference between the $\delta^{15}N$ of the animals and the *Nodularia* is at least +6‰. The samples were collected, however, in winter and spring, which do not coincide with the blooms of *Nodularia*.

In Fig. 2 the isotopic compositions of organisms in Pyramid Lake are displayed on the food web as determined by biological observations (Galat et al. 1981). Upon close inspection the C isotopic compositions and the isotopic fractionations along food chains follow predicted models, but the N isotopic compositions of the fish deviate slightly (3–6‰) from the pattern of progressive enrichment in ^{15}N. With the exception of a few trophic transfers, there is a consistent lack of progressive enrichment up the food chain. For example, the Lahontan cutthroat trout ($\delta^{15}N$ = +9.0) is largely a piscivorous fish as an adult and should have an isotopic composition closer to +15‰ (Sigler et al. 1983).

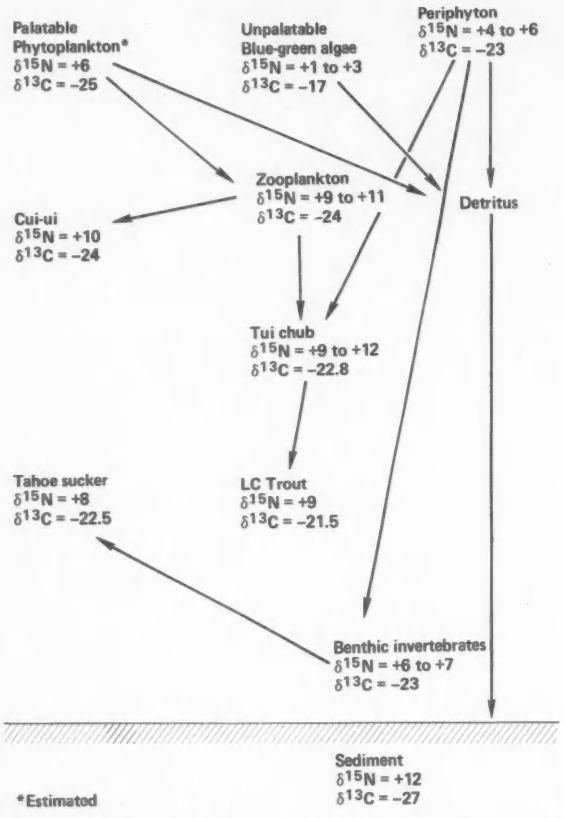

FIG. 2. Relationships of food webs with isotopic compositions of organisms from Pyramid Lake, Nevada.

The chemical and isotopic compositions of muscle from fish are an integration of the diet that the fish has consumed over probably several months, whereas those of short-lived zooplankton probably reflect their diet over less than 1 mo. The light-N isotopic compositions of the fish can be explained by the following. Firstly, in summer, N_2-fixing blue-green algae, although unpalatable, will contribute a portion of isotopically light N ($\delta^{15}N = 0.0$) to the lake. In the autumn, as these algae undergo decay, much of this N is released as inorganic N back into the hypolimnion. During winter and early spring, mixing associated with destratification distributes this light N throughout the water column, and it is thus available for uptake by more palatable species of phytoplankton, the spring diatom pulse (Galat et al. 1981). Secondly, algae growing in N-limited conditions should have isotope ratios nearly identical with that of the source N (Estep and Macko 1985); thus, the $\delta^{15}N$ of Pyramid Lake phytoplankton and zooplankton may vary as the contribution of blue-green algal-fixed N fluctuates. In laboratory experiments, turnover times for $\delta^{13}C$ of 4–20 d have been measured for species of shrimp (Fry and Arnold 1982). Thirdly, the fish living on a diet with a variable isotopic composition will have $\delta^{15}N$ integrated from the extremes over a longer time period. Tieszen et al. (1983) determined that the half-life of $\delta^{13}C$ in muscle tissues of gerbils was 27.6 d. The resulting $\delta^{15}N$ may then be temporal values reflecting the seasonal biogeochemical cycle in contrast with the high C and N turnover by zooplankton.

TABLE 4. Isotopic composition of tui chubs (*Gila bicolor*) sampled inshore and offshore of Pyramid Lake.

Sample description	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
G. bicolor obesa ($n = 6$)		
Average	−22.8	+10.5
Range	−21.9 to −24.6	+8.6 to +12.4
G. bicolor intermediate ($n = 8$)		
Average	−22.9	+11.1
Range	−22.0 to −23.9	+9.8 to +12.6
G. bicolor pectinifer ($n = 5$)		
Average	−22.7	+10.6
Range	−22.3 to −22.9	+9.3 to +11.2
Inshore fish		
February 1983 ($n = 6$)		
Average ±1 SD	−23.1±0.8	+9.9±1.2
Range	−22.5 to −24.6	+8.6 to +11.5
July 1983 ($n = 6$)		
Average ±1 SD	−22.8±0.9	+11.6±0.8
Range	−21.9 to −23.9	+10.4 to +12.6
Offshore fish		
July 1983 ($n = 7$)		
Average ±1 SD	−22.8±0.4	+10.9±0.6
Range	−22.3 to −23.3	+10.0 to +11.5

An example of seasonal variation can be seen in the data of Table 4. The $\delta^{15}N$ of tui chubs collected in February are approximately 2‰ lower than those of samples collected in July from the inshore, but the $\delta^{13}C$ of all the fish are essentially constant. Pyramid Lake has a high concentration of total dissolved inorganic C (1400 ppm) (Galat et al. 1981) that is probably not affected by phytoplankton. Consequently, the algae will fractionate the C isotopes uniformly over the year.

Another aspect of Table 4 is the comparison among the subspecies of *Gila bicolor* (*pectinifer, obesa*, and intermediate). Langdon (1978) showed that *G. bicolor pectinifer* and *G. bicolor obesa* have different diets; Vigg (1983) postulated that this difference in diet was due to differential distribution of gill raker morphs. The inshore fish, primarily *G. bicolor obesa*, consume more benthic invertebrates and periphyton, whereas *G. bicolor pectinifer* consume mainly zooplankton. Fish collected offshore have access only to zooplankton as food and have isotopic compositions reflecting that specific diet. The compositions of the diet are not distinctive enough to differentiate between benthic and planktonic diets. The range of $\delta^{13}C$ and the standard deviations calculated from the two subsets of inshore tui chubs are twice those of the offshore population and may demonstrate the variability in the diet of inshore fish. Fry et al. (1978) determined that intraspecies variability in the $\delta^{13}C$ of a specific population of animals reflects a generalized rather than a specific diet.

Lahontan Reservoir studies

The isotopic compositions of fish from Lahontan Reservoir are presented in Table 5 in relation to trophic level and size. In some species, the generalized diet of the fish changes to include larger prey as the fish reach adult size (Cooper et al. 1983). In general, the larger fish of the species had a heavier isotopic composition than the smaller fish owing to a change in diet. Secondly, an enrichment in the heavy isotopes of both C and N occurs at higher trophic levels. Thirdly, benthic fish have more

TABLE 5. Isotopic composition of fish collected from Lahontan Reservoir, 1983.

Sample No.	Species	Size	Diet[a]	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
		Pelagic			
Fish 14	Sacramento blackfish	Small	Z, A	−21.9	+9.6
Fish 46	(*Orthodon microlepidotus*)	Large	Z, A	−20.6	+9.5
Fish 21	Carp	Small	O, A, P	−22.9	+10.9
Fish 25	(*Cyprinus carpio*)	Large	O, A, P	−21.2	+11.6
Fish 32	Yellow perch (*Perca flavescens*)	Small	Z, F	−22.0	+11.2
Fish 11	Crappie	Small	Z, F	−25.9	+11.8
Fish 35	(*Pomoxis* spp.)	Large	F	−20.4	+13.6
		Benthic			
Fish 45	Tahoe sucker (*Catostomus tahoensis*)	Large	A, I	−16.8	+13.1
Fish 37	Channel catfish (*Ictalurus punctatus*)	Large	I, F, S	−23.6	+13.0
Fish 39	White catfish (*Ictalurus catus*)	Large	O, F	−25.9	+16.2

[a]Z, zooplankton; O, omnivorous; I, invertebrates; A, algae; P, vascular plants; F, fish; S, scavenger.

positive $\delta^{15}N$ values than pelagic fish. An isotope effect in the microbiological decay of organic matter results in the initial release of isotopically light N as ammonia, and the residual organic matter becomes isotopically heavier (Macko and Estep 1985). At the bottom of the lake a pool of isotopically heavy organic N accumulates, and when finally released by microbes is incorporated by periphyton, grazing invertebrates, and finally, fish. Fish feeding on the bottom such as catfish could also be consuming decaying organisms.

Application to Fishery Studies

Hatchery studies

To be useful as tracers, the stable isotopes in the hatchery-reared fish must be different from those in the wild, so that as tissue is replaced or added during maintenance and growth, a change in isotopic composition will record changes in food habits. Hatchery-reared Lahontan cutthroat trout and cui-ui were very similar to their artificial food in isotopic content, and distinct from the wild fish of the same species (Table 6). Carbon isotopes are the most easily measured quantity, and those from wild food are distinct from hatchery food, most likely because of the addition of corn ($\delta^{13}C = -12$) to hatchery food.

Cui-ui larvae are raised first in tanks with only hatchery feed for a diet (Δ49–52 in Table 6). The fish as juveniles are then allowed to feed in artificial outdoor ponds. In the ponds the fish are also able to consume algae and associated invertebrates attached to the walls. The youngest cui-ui had a mean $\delta^{13}C$ value of −19.4, closest to that of their hatchery diet ($\delta^{13}C = -21.2$). The older fish showed a heavier isotopic content: juveniles (101–132 mm) had a mean $\delta^{13}C$ of −18.1, and the larger cui-ui (331 and 321 mm) had a mean value of −17.7. The shift in $\delta^{13}C$ probably corresponds to additions to the diet and may also indicate a change in the metabolism of the fish as it emerges from a juvenile to a mature stage of life. The hatchery-reared animals have quite different $\delta^{13}C$ from the wild samples ($\delta^{13}C$ of −18 vs. −24). The isotopic composition of the wild adult cui-ui ($\delta^{13}C = -24$; $\delta^{15}N = +11$) is nearly identical with that of zooplankton (Table 3). Zooplankton are the postulated food of cui-ui in Pyramid Lake (La Rivers 1962; Koch 1972).

TABLE 6. Isotopic composition of muscle tissue from cui-ui and Lahontan cutthroat trout from hatcheries and the wild of Pyramid Lake.

Sample No.	Sample	Length (mm)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
		Cui-ui		
Δ2	Wild (female)	510	−24.0	+10.2
Δ1	Wild (male)	510	−24.2	+11.5
Δ3	Hatchery (ponds)	321	−18.1	+10.6
Δ4	Hatchery (ponds)	331	−17.4	+10.4
Δ42	Hatchery (2 yr)	132	−18.2	+12.5
Δ43	Hatchery (2 yr)	129	−18.0	+11.6
Δ44	Hatchery (1.5 yr)	101	−18.0	+12.2
Δ45	Hatchery (1.5 yr)	109	−18.2	+11.9
Δ49	Hatchery (juvenile)	37[a]	−19.2	+11.1
Δ50	Hatchery (juvenile)	31[a]	−19.6	+10.7
Δ52	Hatchery (heads)	(from juveniles)	−19.5	+10.4
Δ47	Hatchery feed		−21.2	+7.5
		Lahontan cutthroat trout		
Δ8	Wild		−21.4	+9.2
Δ5	Numana hatchery		−19.8	+9.3
Δ6	Pyramid raceways		−20.0	+8.3
Δ7	Dunn hatchery		−18.3	+6.2
Δ16	Hatchery feed		−18.3	+6.5

[a]Average length of fish in a pooled sample.

The Lahontan cutthroat trout sampled from the Dunn Hatchery was nearly identical, in both the C and N isotopic composition, with the commercial diet it consumed (Table 6). The wild adult trout was clearly different in isotopic composition, and the $\delta^{13}C$ (−21) and $\delta^{15}N$ (+9) correspond very closely to the values reported for the tui chub ($\delta^{13}C = -23$; $\delta^{15}N = +10$), which is the exclusive natural food of large (>300 mm) Lahontan cutthroat trout in Pyramid Lake (Sigler et al. 1983). The isotopic

TABLE 7. Relationship of the isotopic composition of scales to that of muscle from fish from Pyramid Lake and associated hatcheries.

Sample No.	Sample	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
Δ3	Cui-ui muscle	−18.1	+10.6
Δ3S	scales	−15.0	+10.1
Δ4	Cui-ui muscle	−17.4	+10.4
Δ4S	scales	−15.0	+10.2
Δ42	Cui-ui muscle	−18.2	+12.5
Δ42S	scales	−16.1	+10.8
Δ43	Cui-ui muscle	−18.0	+11.6
Δ43S	scales	−15.3	+14.0
Δ44	Cui-ui muscle	−18.0	+12.2
Δ44S	scales	−15.5	+11.7
Δ45	Cui-ui muscle	−18.2	+11.9
Δ45S	scales	−15.4	+10.9
Δ5	Trout[a] muscle	−19.8	+9.3
Δ5S	scales	−14.8	+10.2
Δ18	Tahoe sucker muscle	−22.8	+8.5
Δ18S	scales	−23.2	+7.2
Δ19	Tahoe sucker muscle	−21.9	+8.2
Δ19S	scales	−23.0	+4.1

[a]Lahontan cutthroat trout.

composition of trout from Naumauna Hatchery is anomalous and may reflect a different, moist commercial feed or medicated feed that was used there (A. Ruger, Pyramid Lake Fisheries, Sutcliffe, NV 89510, pers. comm.). Trout reared in Pyramid raceways were intermediate in isotopic composition, although closer to that of the wild fish. The intermediate isotope ratios are logical because the diet of the trout includes both hatchery feed and zooplankton ($\delta^{13}C = -24$; $\delta^{15}N = +10$) present in the continuously circulating Pyramid Lake water supply.

Isotopic composition of fish scales

A comparison between the $\delta^{13}C$ and $\delta^{15}N$ of fish scales and muscle from three species of fish is reported in Table 7. Each species of fish has a characteristic isotope fractionation between scales and muscle tissue. The results of the comparison among cui-ui indicate that there is a consistent 2–3‰ difference in $\delta^{13}C$ among samples of six individual fish. The Tahoe sucker samples show a reverse fractionation, indicating a difference in initial diet or subsequent metabolism. The N isotope patterns are more variable and would not be as valuable a tracer as C isotopes.

Conclusions

Nitrogen isotope ratios have been used to trace very subtle additions and pathways of N in two lakes that are similar in that both are N limited and host large blooms of blue-green algae. The lakes are dissimilar in that Lahontan Reservoir (pH 7.0–9.0) has a proportionally large addition of N from the polluted Truckee Canal and consists of freshwater, whereas Pyramid Lake (pH 9.2) is relatively unpolluted and considerably more saline and alkaline. These differences in chemistry are expressed in the isotopic composition of the organisms living in the lake. The uptake of other available combined forms of N into blue-green algae that are also fixing N_2 is probably occurring in Lahontan Reservoir. Previously, all other $\delta^{15}N$ measurements of N_2-fixing blue-green algae were approximately 0.0‰; hence, analysis of the results presented in this paper would urge caution in either dismissing or overestimating the importance of N_2 fixation based on $\delta^{15}N$ or biological measurements alone. In Pyramid Lake, by examining the $\delta^{15}N$ and $\delta^{13}C$ of the many aspects of the food web, one can see that even though blue-greens are unpalatable, their fixed N eventually enters the food chain after mineralization and recycling.

The C isotope ratios are a further example of how the chemistry of the lake and the physiology of the organisms can be examined by isotopes. The $\delta^{13}C$ of blue-green algae from Lahontan Reservoir was very negative (−26 to −30‰) (Table 2) compared with that of the sample from Pyramid Lake ($\delta^{13}C = -17$) (Table 3). Increased salinity slows the diffusion of CO_2 into plants, creating CO_2 limitation, which results in less isotope fractionation (Guy et al. 1980). Conversely, the $\delta^{13}C$ of the total CO_2 in each of the lakes may be different. A sample of blue-green algae (*Nodularia*) from Walker Lake, also a remnant of pluvial Lake Lahontan, had a $\delta^{13}C$ of −24. The divergence of $\delta^{13}C$ in such similar organisms could be useful in studying the physical and chemical properties of freshwater lakes (Estep 1984).

Because the relationship between the isotopic composition of an animal and its diet have been investigated in various studies, deviations in these intercomparisons can be useful in determining other sources of nutrients in the animal's diet that cannot be seen from direct observation. These deviations can be expressed in the following forms. Firstly, temporal variations within a species, e.g. the $\delta^{15}N$ of inshore tui chubs (Table 4), reflect the different pools of inorganic nutrients and the differences in dietary species compositions, which occur during the yearly biogeochemical cycle. Secondly, variability within a population at one time, e.g. inshore tui chubs, is indicative of a generalized rather than a specific diet. Thirdly, excessive or nonexistent enrichment with trophic level, e.g. the $\delta^{15}N$ of Lahontan cutthroat trout (Fig. 2), suggests the addition of an unsuspected component in an animal's diet. In this case, the component in the diet was most likely tui chubs having a different isotopic composition owing to seasonal variations in the N source. The application of stable isotopes as tracers in aquatic systems thus becomes more powerful as a tool for investigating food webs.

A final application of stable isotopes in determining recruitment of fish from natural reproduction compared with artificial propagation has been evaluated. Stable C isotopes of muscle tissue distinguish a hatchery-reared fish from one grown in the wild. Furthermore, the consistent difference between the $\delta^{13}C$ of muscle tissue and that of scales from the same fish could become a very useful, nondestructive tool for tracing the fate and growth of hatchery-reared fish after they have been released. Different hatcheries could simply use fish food with a different isotopic composition as a biochemical tag so that techniques in hatchery practices could also be evaluated. A very slight artificial enrichment with ^{13}C-labeled compounds would be feasible. Carbon isotope ratio measurements can be determined commercially at a reasonable cost from a number of laboratories, thus facilitating the use of stable isotopes as tracers for fishery management agencies.

In summary, trophic dynamics in a lacustrine system in Nevada have been studied with stable isotope tracers. At the primary trophic level, isotopically heavy N entering Lahontan

Reservoir, possibly from the Reno–Sparks sewage plant, is an important nutrient for the growth of blue-green algal blooms. The N in blue-green algae apparently enters the food web after they have decayed, and the N fixed by these organisms enters the water column as ammonia, which is then incorporated by palatable phytoplankton. At higher trophic levels, analyses or observation of gut content can determine the diet in an animal only at a specific time and cannot determine digestive efficiencies or metabolism. Stable isotope ratios of animals, however, are integrative values reflecting the complexities in feeding habits. Variations in the natural abundance level of C and N isotopes within either wild or hatchery-reared organisms yield information on seasonal variations in diets, generalized versus specific feeding, and species-specific assimilation and metabolic differences.

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Prey Selection by Freshwater Predators with Different Foraging Strategies

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Cooper, S. D., D. W. Smith, and J. R. Bence. 1985. Prey selection by freshwater predators with different foraging strategies. Can. J. Fish. Aquat. Sci. 42: 1720–1732.

We observed several freshwater predators, including the odonate larvae *Pachydiplax longipennis* and *Anax junius*, the hemipterans *Notonecta unifasciata* and *Buenoa scimitra*, the dytiscid larva *Acilius semisulcatus*, and juvenile *Gambusia affinis*, feeding on a variety of microcrustacean prey and determined the frequency of the component parts of predator–prey interactions (encounter, attack, capture, ingestion). Encounter rates were the most important determinant of predator selectivity when predators were presented with a variety of microcrustacean prey. When only copepod species were used as prey, however, both encounter rates and capture success were important in determining predator diets. We used our data to test hypotheses concerning relationships between predator foraging mode and patterns of prey selection: mobile predators exhibited stronger selection for sedentary prey than did sit-and-wait predators; our own and literature data also indicated that macroinvertebrate sit-and-wait predators are better able to capture, and have higher selectivity for evasive prey than do mobile predators. A predator's attack acceleration, however, may be a better predictor of its selectivity for evasive versus nonevasive prey than its mean swimming speed.

Les auteurs ont observé plusieurs prédateurs dulçaquicoles, y compris les larves d'Odonates *Pachydiplax longipennis* et *Anax junius*, les Hémiptères *Notonecta unifasciata* et *Buenoa scimitra*, la larve de Dytiscidés *Acilius semisulcatus* et des juvéniles de *Gambusia affinis*, se nourrissant d'une variété de microcrustacés et ont déterminé la fréquence des composantes des interactions prédateur–proie (rencontre, attaque, capture, ingestion). Les taux de rencontre déterminaient principalement le choix des prédateurs quand ceux-ci étaient en présence d'une variété de proies. Toutefois, quand seulement des copépodes étaient utilisés comme proies, les taux de rencontre et le succès de capture déterminaient en grande partie le régime des prédateurs. Les auteurs ont utilisé leurs données pour vérifier des hypothèses sur les relations entre la méthode de recherche de la nourriture observée chez le prédateur et les systèmes de choix des proies : les prédateurs mobiles choisissaient plus souvent des proies sédentaires que ceux qui étaient à l'affût. De plus, leurs données et celles qui sont déjà publiées révèlent que les prédateurs macroinvertébrés à l'affût ont plus de succès pour la capture et montrent une sélectivité plus élevée des proies fuyantes que les prédateurs mobiles. La vitesse d'attaque de la part du prédateur peut toutefois donner une meilleure prévision de sa sélectivité pour les proies fuyantes par rapport aux proies sédentaires que la vitesse natatoire moyenne.

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An extensive literature is devoted to patterns of prey selection by planktivores (see reviews in Hall et al. 1976; Zaret 1980a; Hurlbert and Mulla 1981; Greene 1983). Much of this work, however, relies on inference and speculation from gut analyses and predation trials to explain patterns in predator diets. Dissection of a predation event into its component parts (encounter, attack, capture, and ingestion), on the other hand, provides direct information about the mechanisms underlying patterns of prey selection (Holling 1966; Swift and Fedorenko 1975; Pastorok 1981; Greene 1983; Riessen et al. 1984; Wright and O'Brien 1984). Specifically, observations of predator and prey behavior permit a better understanding of why and how predators select prey. In addition, direct observation contributes to the testing and refinement of general models of predation (O'Brien 1979; Greene 1983; Riessen et al. 1984; Wright and O'Brien 1984), and may identify the characteristics of prey and predator that allow or preclude their coexistence in nature (Kerfoot 1982).

The specific goals of our work were twofold: (1) to determine the relative importances of various behavioral components (i.e. encounter, attack, capture, and ingestion) to the prey preferences shown by common freshwater predators; (2) to use these behavioral data to test two predictions: (a) Sedentary prey will compose a smaller proportion of the diets of sit-and-wait predators than of the diets of cruising predators. This prediction arises from considerations of the frequency of predator–prey encounter; sit-and-wait predators will only rarely contact and eat sedentary prey (Schoener 1971; Gerritsen and Strickler 1977; Pianka 1982; Greene 1983). (b) Evasive prey will compose a smaller proportion of the diets of active foragers than of the diets of sit-and-wait predators, because evasive prey should be able to detect the approach of a cruising predator and

TABLE 1. Summary of predation experiments. "Predator starvation" is the amount of time predators were starved, and "volume" is the volume of water in which trials were run. TE is the total number of encounters observed and TI is the total number of ingestions observed. NR = not recorded.

Exp.	Predator	Predator starvation (d)	No. of prey types	No. ind./prey type	No. rep. trials	Volume (L)	Duration (min/rep.)	TE	TI
Series I									
1	*Buenoa*	0–1	14	10	9	2	45–170	294	109
2	*Acilius*	NR	13	10	2	2	80–105	192	40
3	*Anax*	1	11	10	8	1	23–65	105	64
4	*Pachydiplax*	5–6	10	10	10	0.5	15–60	158	100
5	*Gambusia*	1	4	10	10	2	1.5–7	220	107
6	*Notonecta*	1	5	10	6	1	60–120	143	47
7	*Notonecta*	4–7	3	20	9	0.5	25–66	100	64
Series II									
8	*Gambusia*	1	2	10	9	2	5	124	17
9	*Pachydiplax*	1	2	40	7	0.5	5–30	121	66
10	*Buenoa*	4	3	20	7	1	15–68	356	43
11	*Gambusia*	2	3	20	7	2	10–11	420	77
12	*Pachydiplax*	5	3	20	8	0.5	20–50	223	68

take evasive action before or early in an attack. Ambush predators do not produce such early-warning signals and should, therefore, more successfully capture evasive prey.

We used several locally available microcrustacean prey in these predation trials, including *Daphnia pulex*, *Ceriodaphnia reticulata*, *Sida crystallina*, *Simocephalus vetulus*, *Scapholeberis kingii*, *Pleuroxus denticulatus*, *Diaptomus pallidus*, *Diaptomus franciscanus*, *Cyclops vernalis*, and ostracods. The predators studied were the larvae of the odonates *Pachydiplax longipennis* and *Anax junius*, the larvae of the beetle *Acilius semisulcatus*, the hemipterans *Notonecta unifasciata* and *Buenoa scimitra*, and the teleost *Gambusia affinis*.

Methods

With the exception of *Acilius* larvae, predators and prey used in these experiments were collected from ponds and lakes in Santa Barbara County, California. *Acilius* larvae were collected from North Inyo Crater Pond in Mono County, California. Predation trials were conducted in the laboratory in small aquaria (25.5 × 6.4 × 15.2 cm, internal $l \times w \times h$) under constant illumination from fluorescent light sources. Water temperatures ranged from 22 to 27°C. Other experimental conditions are listed in Table 1.

We introduced equal numbers of each of 2–14 prey types into aquaria containing filtered (52 μm) pond water. Container volumes and prey densities were manipulated so that we could observe a number of interactions in 1 h. Predators were added at the start of the experiment, and the behavior of the animals was watched for either a predetermined time (0.5–2 h) or until a predetermined number of prey had been eaten (usually 10). We introduced a different predator individual for each replicate trial, and individual predators were never reused.

We noted the number of encounters (E), attacks (A), captures (C), and ingestions (I) and measured handling times (HT) for each prey type, and then calculated the number of predator–prey encounters per hour, attack probability (proportion of encounters resulting in attack = A/E), capture success (proportion of attacks resulting in capture = C/A), ingestion efficiency (proportion of captures resulting in ingestion = I/C), and α, a selectivity index (Chesson 1978, 1983). We could not, however, count both encounters and attacks for two predators, *Pachydiplax* and *Gambusia*. *Pachydiplax* showed no obvious behavioral responses to prey prior to attack, and in the *Gambusia* trials we could score only predator attacks on prey, but not encounters, because of the high rate of predator–prey interactions. In most trials, prey were replaced as they were eaten; however, in the *Gambusia* trials, prey were consumed too rapidly for replacement. In the *Gambusia* trials, we estimated prey preferences with formulae that take prey depletion into account (Chesson 1983).

Two sets of experiments were conducted. In the first series of predation trials, individuals of each of six predator species were presented with a prey assemblage composed of a variety of cladoceran, ostracod, and copepod species. In the second series of trials, a mixture of two or three copepod species was presented to individual predators. Three different predator species were used in these trials (Table 1).

Swimming speeds of prey were measured after experiments 1, 4, and 12 (Tables 1, 2) under the same conditions as used in those experiments (i.e. predators present). The back and bottom of the aquarium were marked with a grid composed of 1 × 1 cm cells, and two observers traced swimming paths on similarly gridded paper. Individual prey were watched for 1 min, swimming paths were monitored for at least five individuals of each prey type, and traced swimming paths were measured with a map wheel. With the exception of *Acilius* larvae, swimming or crawling speeds of predator individuals were determined, immediately after predation trials, using the techniques outlined above. Prey lengths (exclusive of the tail spine in *Daphnia* and the caudal furca in copepods) and predator head widths were measured with an ocular micrometer at ×6 to ×25, and lengths of predators were measured with a millimetre rule.

We used sign tests to determine if there were consistent differences in encounter rate, attack probability, capture success, ingestion efficiency, and α among prey types for each predator species (Conover 1982). Statistical analyses for trials using *Acilius* are not shown because there was insufficient replication in these trials for statistical testing. We used the Kruskal–Wallis test to determine if there were significant

TABLE 2. Characteristics of predators and prey used in predation trials. Numbers in parentheses under life history stages of some predators are predator head widths in millimetres. Prey swimming speeds (SS) measured after experiments 1, 4, and 12 are presented separately because prey speeds differed significantly among trials. Numbers in parentheses under prey and predator speeds are standard errors. For comparison, we have listed swimming speeds reported in the literature for these prey or similarly sized species in the same genus.

Predators

Species	Life history stage	Total length (mm)	Foraging mode	Foraging speed (mm/s)	Microdistribution	Code
Pachydiplax longipennis	Larva (4.2)	19	Ambush	0	Rests on bottom	P
Notonecta unifasciata	Adult	10	Ambush	0	Rests at surface	N
Anax junius	Larva (4.9)	25	Slow stalk or ambush	0.9	On bottom	An
Buenoa scimitra	Adult	7	Continuous swimming – stroke – glide stroke	4.9 (1.1)	Water column; does not forage at surface or bottom	B
Acilius semisulcatus	Larva (1.5)	22	Continuous swimming	—	Water column; sometimes at surface, not at bottom	Ac
Gambusia affinis	Juvenile	24	Continuous swimming	11.8 (2.6)	Throughout water column	G

Prey

Species	Size class	Length (mm)	SS_1 (mm/s)	SS_4 (mm/s)	SS_{12} (mm/s)	SS_{lit} (mm/s)	Microdistribution	Code
Ceriodaphnia reticulata	Large (l)	0.70–0.78	0.4 (0.2)	1.5 (0.3)	—	0.8–1.5[a]	Water column, but tends to be in corners	Ce
C. reticulata	Small (s)	0.38	—	—	—	—	Water column, but tends to be in corners	Ce
Daphnia pulex	Large (l)	2.1–2.3	1.3 (0.2)	4.9 (0.4)	—	—	Throughout water column	Da
D. pulex	Medium (m)	1.35–1.50	0.9 (0.2)	2.0 (0.5)	—	1.3–3.3[c,d,e]	Throughout water column	Da
D. pulex	Small (s)	0.70–0.82	0.7 (0.1)	0.8 (0.1)	—	0.5–1.2[a,c]	Throughout water column	Da
Scapholeberis kingii	Large	0.60–0.69	0.07 (0.03)	—	—	—	Associated with surface film	Sc
Pleuroxus denticulatus	Large	0.40–0.60	0.0	0.5 (0.2)	—	—	Attached to or crawling on sides or bottom, especially in corners	Ch
Ostracod	Small	0.61–0.73	0.03 (0.02)	0.5 (0.4)	—	—	Moving slowly or stationary on bottom	O
Sida crystallina	Large	1.22	0.0	—	—	—	Attached to sides or bottom	Sd
Simocephalus vetulus	Large (l)	1.78–1.90	0.1 (0.1)	0.0	—	—	Attached to sides or bottom; swims into water column very infrequently	Sm
S. vetulus	Medium (m)	1.32	0.2 (0.2)	—	—	—	Attached to sides or bottom; swims into water column very infrequently	Sm
S. vetulus	Small (s)	0.65–0.82	—	0.8 (0.5)	—	—	Attached to sides or bottom; swimns into water column very infrequently	Sm
Cyclops vernalis	Large	0.96–1.60	0.0	0.9 (0.4)	9.2, 0.4[h] (2.8, 0.2)	1.5–3.0[a,b]	Usually resting, occasionally swimming on bottom; sometimes on sides, occasionally in water column	Cy

TABLE 2. (*Concluded*)

Species	Size class	Length (mm)	Prey SS_1 (mm/s)	SS_4 (mm/s)	SS_{12} (mm/s)	SS_{lit} (mm/s)	Microdistribution	Code
Diaptomus pallidus	Small	1.00–1.48	0.5 (0.1)	1.0 (0.2)	1.1, 1.2[h] (0.5, 0.1)	0.2–1.5[a,c,d,f,g]	Throughout water column	Di
Diaptomus franciscanus	Large	1.67–2.02	0.7 (0.1)	—	1.9, 4.3[h] (0.6, 0.6)	2.4–3.2[g]	Throughout water column	Di

[a]Li and Li 1979.
[b]Gerritsen 1978.
[c]Gerritsen 1980a.
[d]Gerritsen 1980b.
[e]Pastorok 1980b.
[f]Swift and Fedorenko 1975.
[g]Giguére et al. 1982.
[h]Values for Experiment 12. The first value is mean swimming speed for the first five replicate trials and the second is mean swimming speed for the last three trials.

effects of predator species on the encounter probabilities, attack probabilities, capture successes, ingestion efficiencies, and αs for each prey type. If this test indicated significant differences among predator species for a given behavioral parameter for a given prey species, we applied the multiple comparisons procedure (Conover 1982). An encounter probability, $p(E)$, for a given prey type is the encounter rate for that prey type divided by the total encounter rate for all prey types under consideration. For comparisons among predator species we only used data for the prey types common to all experiments, i.e. large *Daphnia pulex*, large *Simocephalus vetulus*, adult *Diaptomus pallidus* females, and adult *Cyclops vernalis* females in the series I trials and adult *Diaptomus pallidus* females, adult *D. franciscanus* females, and adult *Cyclops vernalis* females in the series II trials. Other statistical methods were taken from Conover (1982).

Results

Characteristics of Predators and Prey

The characteristics of predators and prey used in these experiments are summarized in Table 2. The predators can be ranked along a gradient of average speed as *Pachydiplax* < *Notonecta* < *Anax* < *Buenoa* < *Acilius* < *Gambusia*. Although *Pachydiplax* and *Notonecta* are both sit-and-wait predators, *Notonecta* changed its perch site more often than *Pachydiplax*. *Anax* larvae used a mixed strategy, alternately crawling and remaining motionless, while *Buenoa*, *Acilius*, and *Gambusia* moved more or less continuously. *Notonecta* and *Pachydiplax* generally respond only to very close prey, but the other predators responded to and stalked prey over distances of >2 cm.

The prey used in these predation trials display a variety of behavioral characteristics (Table 2). The pelagic zooplankton (*Daphnia*, *Ceriodaphnia*, and the calanoid copepods) swim continuously, and swimming speeds increase with increasing body size. The ostracods, littoral cladocerans (*Sida*, *Simocephalus*, *Scapholeberis*, *Pleuroxus*), and cyclopoid copepods are more sedentary. The copepods exhibit much more vigorous escape responses than the cladocerans or ostracods (Drenner et al. 1978).

Prey Preferences and Behavioral Observations

In the first series of predation trials, we simultaneously exposed each predator to a variety of microcrustacean prey (Table 1). Encounter rate was, by far, the most important determinant of predator diets in these trials (Fig. 1, 2, 3, 4). For all predator species, there were significant positive correlations between encounter rate and selectivity (α) among prey types (Spearman rank correlation coefficients (r_s) = +0.70 to +0.98, $P < 0.10$–0.001). For the invertebrate predators, there were no significant differences in capture success among different sizes and species of ostracod or cladoceran prey (Table 3; Fig. 2–4). All predators ingested virtually all prey that they captured; consequently, there were also no significant differences in ingestion efficiency among different sizes and types of prey. Thus, patterns of prey selection among cladoceran and ostracod prey were determined almost entirely by encounter rates. All invertebrate predators preferred large over small *Daphnia* because encounter rates increased with increasing daphnid size (Fig. 1, 3). Encounter rates and selectivity indices were generally low for small littoral cladocerans (*Sida*, small *Simocephalus*, *Pleuroxus*, *Scapholeberis*) compared with large pelagic cladocerans (i.e. *Daphnia*, Fig. 1, 4). The low encounter rates for small littoral cladocerans can be attributed to at least three factors: (1) these prey were small and, consequently, inconspicuous to the large-size selective predators used in these experiments; (2) these prey moved slowly or infrequently, thereby reducing encounter rates with sedentary predators such as *Pachydiplax*; (3) these benthic or surface prey were microspatially segregated from predators which foraged in the water column. An exception to the latter pattern was *Notonecta*'s relatively high α for *Scapholeberis*, but *Notonecta* occurred in the surface stratum where *Scapholeberis* was found, and often encountered and ate this small cladoceran. For most predators, capture success for cladocerans was generally high, compared with copepods, and did not vary much among species or prey sizes (Table 3; Fig. 2, 3, 4). Capture successes for copepods, on the other hand, were often low and differed among copepod species (see below, Table 3; Fig. 2, 4, 5).

A second series of experiments (series II) was conducted to

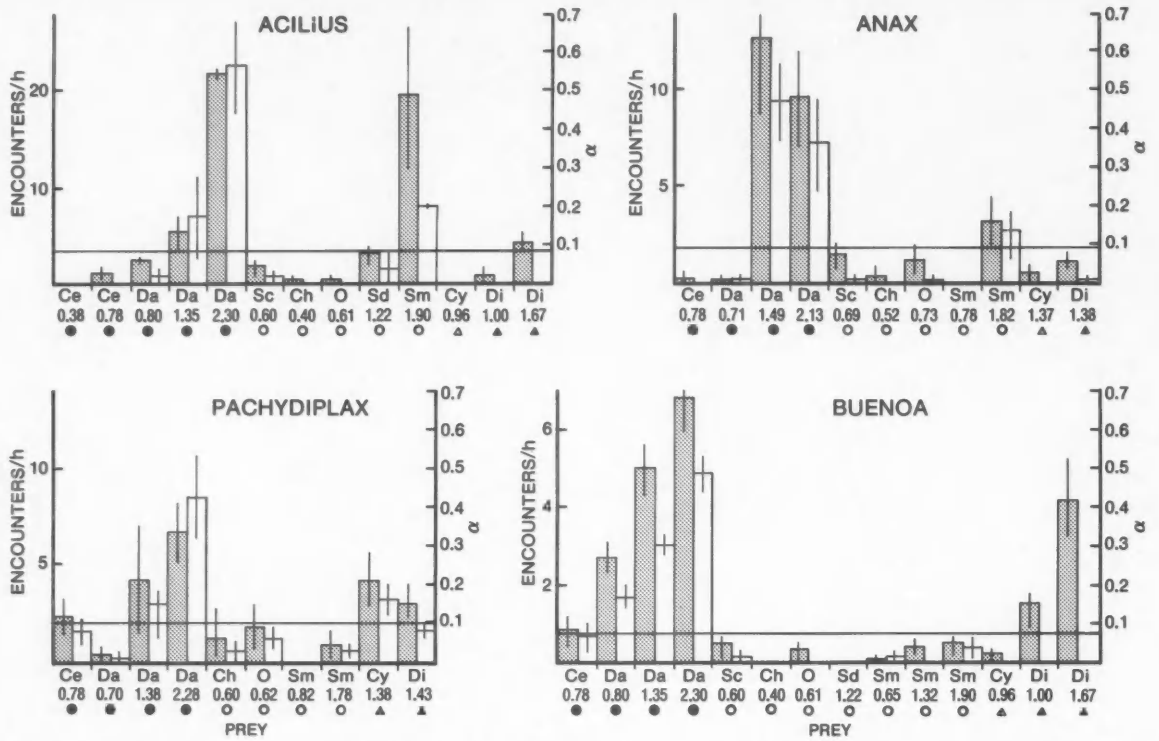

FIG. 1. Encounter rates (encounters per hour, stippled bars) and selectivity indices (αs, open bars) for prey types presented to each of four predator species. Numbers under each prey type are prey lengths in millimetres. Solid circles denote pelagic cladocerans, open circles denote littoral cladorcerans and ostracods, solid triangles signify cyclopoid copepods, and open triangles denote calanoid copepods. Prey code is presented in Table 2. The horizontal line represents the value if selection were random. Vertical bars = ±1 SE.

further study predation by *Gambusia*, *Buenoa*, and *Pachydiplax* on evasive, copepod prey. In these experiments, the relative importance of encounter rate and capture success in determining predation rates was dependent on the particular combination of predator and prey. When *Gambusia* was used as a predator (experiment 8), electivity indices for *Diaptomus pallidus* and similarly sized (1.25 mm) *Cyclops vernalis* were not significantly different ($\bar{X} \pm 1\,SE = 0.43 \pm 0.15$ vs. 0.57 ± 0.15). In this experiment, higher encounter rates for *Diaptomus* were effectively balanced by higher capture success for *Cyclops* (Di $A/h = 117.3 \pm 36.9$, $C/A = 0.09 \pm 0.03$ vs. Cy $A/h = 48.0 \pm 12.5$, $C/A = 0.38 \pm 0.12$; $P < 0.05$, Wilcoxon signed ranks test). With *Pachydiplax* as the predator, encounter rates and αs for *Cyclops* were significantly higher than those for *Diaptomus* ($\bar{X} \pm 1\,SE = 68.0 \pm 19.9$ A/h, $\alpha = 0.72 \pm 0.09$ for *Cyclops* vs. 24.7 ± 7.3 A/h, $\alpha = 0.28 \pm 0.09$ for *Diaptomus*, $P < 0.05$, Wilcoxon signed ranks test). There were no differences in *Pachydiplax* capture success for these two prey species (Cy $C/A = 0.57 \pm 0.07$, Di $C/A = 0.53 \pm 0.13$, experiment 9). Thus, these results support the hypothesis that ambush predators can more efficiently capture evasive prey, such as *Diaptomus*, than can mobile predators (see below).

When presented with three copepod species (experiments 10–12), all three predators had low capture successes and electivity values for the large diaptomid (*Diaptomus franciscanus*) in spite of high encounter and attack rates for this prey type (Fig. 5). *Gambusia* preferred *Cyclops* over both diaptomids, primarily because of the fish's higher capture success for cyclopoids (Table 3; Fig. 5). *Buenoa*, on the other hand, selected the smaller diaptomid, *Diaptomus pallidus* over the other two copepods, because this predator rarely encountered the sedentary, benthic cyclopoids and could not capture *Diaptomus franciscanus*.

In the *Pachydiplax* trials, there was an abrupt change in cyclopoid behavior between the fifth and sixth trials (Table 2). During the first five trials, cyclopoids were quite active, swimming at an average speed of 9.2 mm/s, while in the last three trials, the cyclopoids became more quiescent (average swimming speed of 0.4 mm/s). As a result, encounter rates and electivity indices for cyclopoids were much higher in the first five trials than in the last three (trials 1–5: Cy $A/h = 31.4 \pm 5.9$, $\alpha = 0.75 \pm 0.07$; trials 6–8: Cy $A/h = 2.9 \pm 1.0$, $\alpha = 0.15 \pm 0.08$, $P < 0.05$, Mann–Whitney *U*-test). Encounter rates and capture success for both species of *Diaptomus*, and capture success for *Cyclops*, did not significantly differ between the first five versus the last three trials. Because *Cyclops* became much rarer in the diet of *Pachydiplax* in the last three trials, electivity indices for *Diaptomus pallidus* increased (trials 1–5: $\alpha = 0.17 \pm 0.05$; trials 6–8: $\alpha = 0.72 \pm 0.17$; $P < 0.05$, Mann–Whitney *U*-test). These results emphasize the importance of prey activity and consequent encounter rates in determining the diets of ambush predators. In the series I and II trials, ingestion efficiency was similar for all prey types and did not influence patterns of predator selectivity.

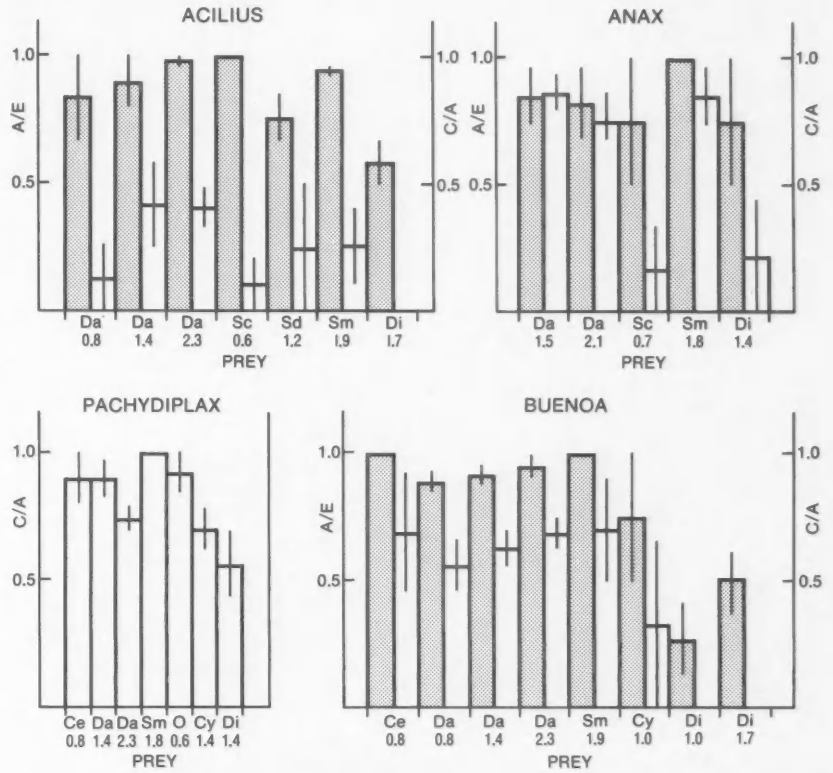

FIG. 2. Attack probabilities (A/E, stippled bars) and capture successes (C/A, open bars) for prey types presented to each of four predator species. Other designations as in Fig. 1.

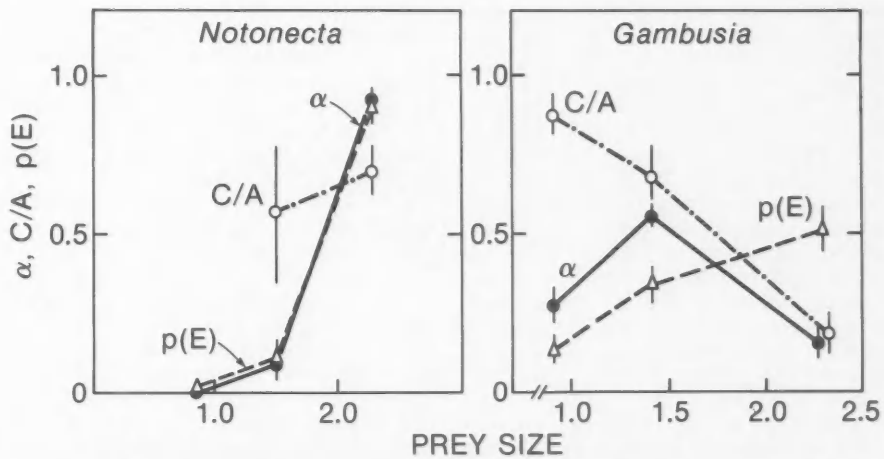

FIG. 3. Encounter probabilities ($p(E)$s, open triangles), capture successes (C/A, open circles), and selectivity indices (αs, solid circles) for different sizes of *Daphnia pulex* presented to *Notonecta* (experiment 7). For comparison we included data on *Gambusia* size selectivity taken from Bence and Murdoch (1985). Vertical bars = ±1 SE.

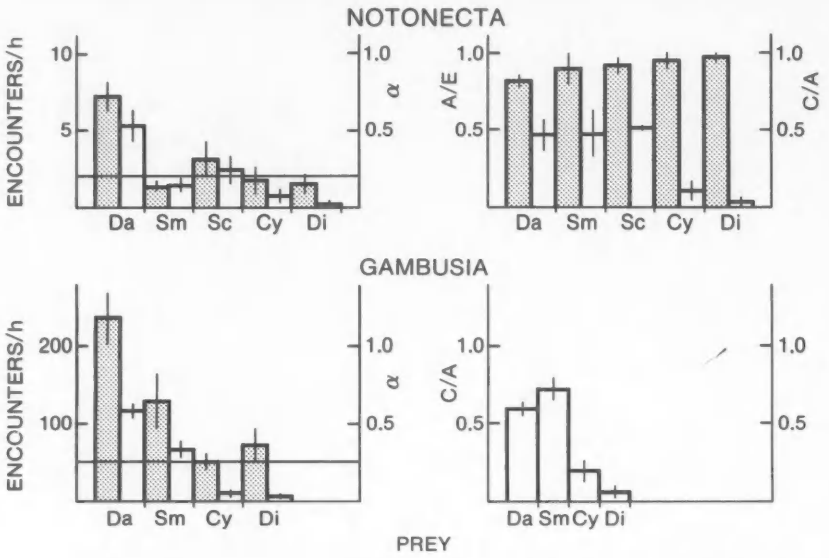

FIG. 4. Left: encounter rates (encounters per hour, stippled bars) and selectivity indices (αs, open bars) for prey types presented to *Notonecta* and *Gambusia*. Right: attack probabilities (*A*/*E*, stippled bars) and capture successes (*C*/*A*, open bars) for prey types used in these trials. Other designations as in Fig. 1. Mean lengths of prey used in these trials were as follows: *Notonecta* trials: Da = 1.52 mm, Sm = 1.51 mm, Sc = 0.58 mm, Di = 1.21 mm, and Cy = 1.18 mm; *Gambusia* trials: Da = 1.68 mm, Sm = 1.69 mm, Di = 1.35 mm, and Cy = 1.37 mm.

Test of the Hypotheses

We first tested whether selection for sedentary prey is stronger by cruising than by ambush predators. To do this we examined selectivity for *Simocephalus* (sedentary prey) over similarly sized *Daphnia* (mobile prey) by using data for only these two prey types. The results support our hypothesis: there was a significant positive correlation between average predator foraging speed and the electivity index for *Simocephalus* (Spearman $r_s = +0.83$, $P < 0.05$). This result was the product of a positive correlation between encounter probability for *Simocephalus* and predator foraging speed (Spearman $r_s = +0.60$, $P < 0.10$). There was no relationship between predator foraging speed and capture success for *Simocephalus* (Spearman $r_s = -0.43$, $P > 0.10$). Encounter rates and electivity indices for large *Simocephalus* were generally low for *Buenoa* and the ambush predators (*Notonecta*, *Pachydiplax*), but were higher for visual cruising predators (*Acilius*, *Gambusia*, *Anax*) (Table 4; Fig. 1, 4). Although they sometimes adopted an ambush foraging mode, we classified *Anax* naiads as cruising predators because they were often observed stalking large *Simocephalus* over distances of several centimetres.

Second, we tested whether selection for evasive prey is stronger by ambush than by cruising predators. We did this by comparing our results for evasive prey (the copepod *Diaptomus*) with those obtained for comparably sized, nonevasive *Daphnia*. Adult copepods have much stronger escape responses than *Daphnia* (Drenner et al. 1978), while other important characteristics (microdistributions and swimming speeds of *Daphnia* and *Diaptomus*) were comparable in our experimental containers. Although all predators consumed more *Daphnia* than copepods, the relative rates at which these two prey types were eaten corresponded to our prediction. Among invertebrate predators, there was a significant negative correlation between predator foraging speed and α for *Diaptomus* ($r_s = -0.93$, $P < 0.01$), and between predator foraging speed and capture success for *Diaptomus* ($r_s = -0.83$, $P < 0.05$). There was no relationship between predator foraging speed and encounter probability for *Diaptomus* ($r_s = -0.20$, $P > 0.10$). However, when the vertebrate predator, *Gambusia*, was included in the analyses, there were no significant correlations between predator foraging speed and αs, encounter probabilities, or capture successes for copepods ($r_s = -0.50$ for predator speed vs. *C*/*A*, $r_s = -0.39$ for predator speed vs. α). This change can be attributed to *Gambusia*'s slightly higher capture success for copepods when compared with cruising invertebrate predators (see Discussion). With one exception, capture successes and electivity indices for copepods were significantly higher for our most sedentary predator, *Pachydiplax*, than for other predator species in the first series of trials (Fig. 1, 2, 4; Table 4). In the one exception, capture success for *Diaptomus* was not significantly different for *Pachydiplax* versus *Anax*. Capture success for *Diaptomus* was, however, generally low and variable in the *Anax* trials (Fig. 2).

There was a significant positive correlation between attack probability and capture success across prey types for *Buenoa* and *Anax* (*Buenoa* = +0.99, $P < 0.001$; *Anax* = +0.88, $P < 0.05$; Spearman rank correlation analysis). Although these correlations could be interpreted as active predator choice of nonevasive prey, our observations suggest that these correlations were a consequence of prey flight from approaching predators before an attack was launched.

To test the generality of our hypothesis regarding the relationship between the foraging mode of macroinvertebrate predators and selectivity for evasive prey, we combined our data with literature data. In our survey we examined results from labora-

TABLE 3. Summary of significant differences in behavioral parameters among prey types for each predator species. Prey types connected by an underline are not significantly different ($P > 0.05$, sign test). Because of the limited number of replicates, we could not run statistical tests for the *Acilius* trials. Tests dealing with ingestion efficiency are not presented because ingestion efficiencies were not significantly different among prey types. Prey code and sizes are given in Table 2. The letters l, m, and s after the prey code designates size classes large, medium, and small. IR = insufficient number of values for a statistical test; α = selectivity index, E = encounter rate, A/E = attack probability, and C/A = capture success.

Predator	Exp.	Parameter	High → Low
Buenoa	1	α	Dal Dam Das Ce Sml Sc Sms Smm Sd Ch O Dil Dis Cy
		E	Dal Dil Dam Das Dis Sml Ce Smm Sc O Cy Sd Chy Sms
		A/E	Dal Dam Das Sml Dis Dil
		C/A	Dal Dam Das Sml Dil
Anax	3	α	Dam Dal Sml Sc Di Das O Ce Sms Chy Cy
		E	Dam Dal Sml Sc Di O Cy Chy Ce Das Sms
		A/E	Dam Dal
		C/A	IR
Pachydiplax	4	α	Dal Cy Dam Ce O Di Sml Chy Das Sms also Cy > O, Di > Das
		E	Dal Di Cy Dam Ce O Chy Das Sml Sms also Dam > Das, Cy > Ce
		C/A	Dal Cy Di
Gambusia	5	α	Da Sm Di Cy
		E	Da Sm Di Cy
		C/A	Sm Da Cy Di
Notonecta	6	α	Da Sc Sm Cy Di
		E	Da Sc Cy Di Sm
		A/E	Da Sc Sm Di Cy
		C/A	Sc Da Sm Cy Di
Buenoa	10	α	Dis Cy Dil
		E	Dil Dis Cy
		A/E	Cy Dis Dil
		C/A	Cy Dis Dil
Gambusia	11	α	Cy Dil Dis
		E	Dil Dis Cy
		C/A	Cy Dil Dis
Pachydiplax	12	α	Dis Cy Dil
		E	Dil Cy Dis
		C/A	Cy Dis Dil

tory or in situ studies where predators were exposed to copepods (usually *Diaptomus*), cladocerans (usually *Daphnia* of comparable size), or both together. In some studies, additional prey types were available. We also included the results from a few field diet studies where prey abundance had been quantified. We assumed that relative predation rates on copepods versus daphnids were not affected by whether copepods and daphnids were available alone, together, or with additional prey types. For the statistical analysis of these data, we followed the conservative procedure of treating each predator genus as a replicate. Ambush predators had significantly higher electivity indices for copepods (over cladocerans) than did cruising predators (Table 5).

Discussion

Our observations of predator–prey interactions illustrate several important points. First, except for copepods, prey were virtually defenseless against predators. Cladocera, especially, were attacked, captured, and eaten when encountered; thus, their coexistence with predators in nature must rely on mechanisms (temporal or spatial segregation, small size, or reduced visibility) that minimize the frequency of encounters with predators (Greene 1983). Copepods, on the other hand, were often able to evade capture (Drenner et al. 1978; Wright and O'Brien 1984). Second, we found that predators attacked nearly

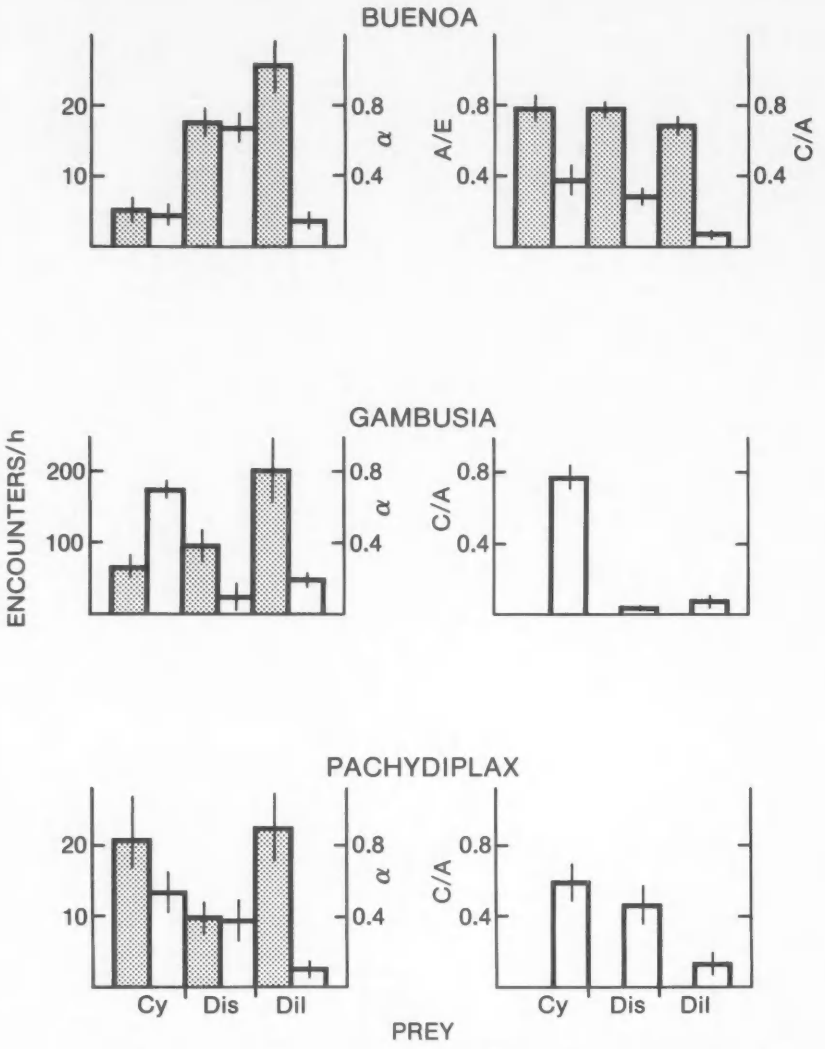

FIG. 5. As for Fig. 4 but for three copepod species presented to each of three predator species. Random selection of these three prey types would result in selectivity indices (αs) of 0.33. Prey code and lengths: Cy = *Cyclops vernalis*, 1.60 mm; Dis = *Diaptomus pallidus*, 1.48 mm; and Dil = *D. franciscanus*, 2.01 mm.

all prey that they encountered; the preferences demonstrated by these predators were largely a function of the prey's vulnerability to encounter and capture. Thus, many of the predators examined here did not appear to "choose" to attack prey. Although ingestion efficiency played little role in determining patterns of prey selection by predators in our experiments, ingestion efficiency can play a role in determining predation rates on hard-bodied (e.g. large ostracods) or bad-tasting prey (e.g. water mites) by some of these predators (Kerfoot et al. 1980; Kerfoot 1982; S. D. Cooper, D. W. Smith, and J. R. Bence unpubl. data).

We predicted that mobile predators would select sedentary prey more strongly than would ambush predators. This prediction stems from a consideration of predator–prey encounter frequencies: cruising predators should encounter sedentary prey at higher rates than do ambush predators (Gerritsen and Strickler 1977). Indeed, our results confirmed that ambush predators rarely encountered, and therefore rarely ate, sedentary prey. In contrast, sedentary prey were more vulnerable to active predators because of higher encounter rates with these predators. An exception to this trend was *Buenoa*, a cruising predator whose electivity index for sedentary prey (i.e. *Simocephalus*) was low; however, *Buenoa* rarely responded to, and did not attack, prey that were not actively swimming in the water column.

Data reported in the literature also support the above hypothesis. Active predators, including flatworms, cyclopoid copepods, and some water mites, feed at comparable rates on *Daphnia* and similarly sized *Simocephalus* (Paveljeva and Zankai 1971;

TABLE 4. Summary of significant differences in measured parameters among predator species for each prey type. Predator species connected by an underline are not significantly different ($P > 0.05$, Kruskal–Wallis test, multiple comparisons procedure). Tests dealing with ingestion efficiencies are not presented because ingestion efficiencies were not significantly different among predator species. Predator code as in Table 2; parameter codes as in Table 3.

Prey	Parameter	High	Low
Series I			
Daphnia	α	B Ac N An P G	
	$p(E)$	B An N P Ac G	
	A/E	Ac B An N	
	C/A	An P B G N Ac	
Simocephalus	α	Ac G An N B P	
	$p(E)$	Ac G N An B P	
	A/E	B An N Ac	
	C/A	P An B G N Ac	
Diaptomus	α	P G An N B Ac	
	$p(E)$	P B G N An Ac	
	A/E	N An Ac B	
	C/A	P An G N B Ac	
Cyclops	α	P G N An B Ac	
	$p(E)$	P N G B An Ac	
	A/E	N B An	
	C/A	P B G N An	
Series II			
Large *Diaptomus*	α	G B P	
	$p(E)$	G B P	
	C/A	P B G	
Small *Diaptomus*	α	B P G	
	$p(E)$	B G P	
	C/A	P B G	
Cyclops	α	G P B	
	$p(E)$	P G B	
	C/A	G P B	

Brandl and Fernando 1975; Schwartz and Hebert 1982), and cruising fish and copepod predators feed heavily on stationary *Chaoborus* larvae (Stenson 1978, 1980; Williams 1980; Kerfoot 1982). Some damselfly naiads can apparently switch from an ambush to a cruising foraging mode resulting in increased selection for the sedentary *Simocephalus* relative to *Daphnia* (Akre and Johnson 1979; Johnson and Crowley 1980). We could find no examples of the relative selectivity on mobile versus sedentary prey by ambush predators, although one report (Smyly 1980) suggested that the sedentary habit of *Chydorus* effectively limits its losses to the ambush predator, *Chaoborus*.

Active predators preyed more equitably on sedentary and mobile prey than did ambush predators; however, in the field this pattern is complicated by at least two factors. First, many active predators are open-water forms and they rarely encounter sedentary prey that occur mainly in vegetation or on the bottoms of lakes. Even within our small experimental containers there was microspatial segregation of predators and prey. Second, some cruising predators (e.g. *Buenoa*) may respond to and attack only moving prey. This would be true both for tactile predators which respond only to hydrodynamic cues and to visual predators which respond only to prey motion (Pritchard 1965; Strickler 1975; Pastorok 1980a). In addition, moving prey are more conspicuous than nonmotile prey to those predators that do not require prey movement to stimulate attack (Confer and Blades 1975; O'Brien 1979; Zaret 1980b; Kerfoot 1982; Janssen 1982; Wright and O'Brien 1984). For these reasons, active predators may also take a preponderence of active versus sedentary prey, although the differential should not be as marked as that displayed by sedentary predators.

Our second prediction was that evasive prey would be more vulnerable to sedentary as opposed to mobile predators because capture success would be higher for ambush predators. Analyses of our own and literature data indicated that capture successes and electivity indices for copepods are very low for all macroinvertebrate cruising predators, whereas some ambush predators are relatively efficient at capturing copepods and, hence, have relatively high electivity indices for copepods.

Other, less direct, evidence also supports this conclusion. For example, some studies have reported the efficient capture of copepods by ambush predators, and copepods are often an important component of the diets of such ambush predators as zygopteran naiads, corixids, hydroids, and chaoborids (Fischer 1964; Lawton 1970; Griffiths 1973; Reynolds 1975; Thompson 1978; Kajak and Rybak 1979; Hairston 1979; Cuker and Mozley 1981). In contrast, cruising predators, such as water mites and freshwater jellyfish, have very low predation rates on evasive copepods compared with nonevasive prey (Ellis-Adam and Davids 1970; Dodson and Cooper 1983). Data on the selection of evasive (*Diaphanasoma*, helmeted *Daphnia*) versus nonevasive cladocerans (nonhelmeted *Daphnia*) by cruising (water mites, *Buenoa*, and related forms) and ambush predators (*Chaoborus*) also indicate that electivity indices for evasive prey will be higher for ambush than for active predators (O'Brien and Vinyard 1978; Winner and Greber 1980; Grant and Bayly 1981; Riessen 1982; Cooper and Smith 1982). Among freshwater predators, then, there is a general relationship between predator foraging mode and selectivity for evasive prey.

There are, however, two groups of cruising predators, the predatory copepods and particulate-feeding fish, which often have relatively high electivity values for evasive versus nonevasive prey. In the case of predatory copepods, other parts of the predator–prey interaction (e.g. ingestion efficiency) may override the importance of capture success in determining patterns of prey selection (Kerfoot 1977, 1978; Li and Li 1979; O'Brien et al. 1979, 1980; Williamson and Gilbert 1980; Williamson 1983; Dodson 1984). In the latter case, fish can counter the evasive responses of prey by modifying their attack behavior (Confer and Blades 1975; Vinyard 1980, 1982; Winfield et al. 1983). In fact, some fish switch from a continuous cruising mode to more of an ambush mode when attempting to catch copepods (J. R. Bence, unpubl. data). In contrast with other predators, both fish and copepods will often actively pursue and overtake escaping zooplankton prey (Jamieson 1980; Winfield et al. 1983).

Is there a better predictor than predator foraging speed of a predator's degree of selection for evasive versus nonevasive prey? Although our hypothesis is correct to some extent, it appears that the attack acceleration of predators may be more important than foraging speed in determining the success of predators in capturing evasive prey. Predators that are success-

TABLE 5. Summary of capture successes (*C/A*) and selectivity indices (αs, Chesson 1978, 1983) calculated for evasive versus nonevasive prey for a variety of ambush and active predators. Unless otherwise noted, comparisons are for *Diaptomus* (Di) versus comparably sized *Daphnia* (Da). Selectivity indices for *Diaptomus* were significantly higher for ambush than for cruising predators ($P < 0.05$, Mann–Whitney *U*-test).

Predator	Di α	Di *C/A*	Da *C/A*	Predator species and literature source	Notes
Ambush					
Chaoborus	0.58	0.30	0.44	*flavicans* α: Swüste et al. 1973; Smyly 1980. *C/A*: Smyly 1980	
				americanus α: Vinyard and Menger 1980; Sprules 1972; von Ende and Dempsey 1981; Anderson and Raasveldt 1974; Lynch 1979; Melville and Maly 1981. *C/A*: Vinyard and Menger 1980; Swift and Fedorenko 1975; Riessen et al. 1984	Vinyard and Menger 1980: α = Di vs. *Moina*
				punctipennis α: Winner and Greber 1980	Lewis 1977 and Winner and Greber 1980: α = Di vs. *Diaphanosoma*
				sp. α: Lewis 1977	
				trivittatus C/A: Swift and Fedorenko 1975	
Notonecta	0.16	0.03	0.46	*undulata* α: Lynch 1979	
				unifasciata α and *C/A*: this study	
Pachydiplax	0.40	0.56	0.90	*longipennis*: this study	
$\bar{X}$	0.39	0.30	0.60		
SE	0.13	0.15	0.15		
Cruising					
Mysis	0.09	0.20	0.73	*relicta* α: Grossnickle 1978; Cooper and Goldman 1980; Bowers and Vanderploeg 1982; Ramcharan et al. 1985. *C/A*: Ramcharan et al. 1985	Ramcharan et al. 1985: α = Cy vs. Da
Neomysis	0.20			*mercedis* α: Murtaugh 1981	Murtaugh 1981: α = $\bar{X}$ of field and lab data
Piona	0.02			*constricta* α: Riessen 1982	
				limnetica α: Gliwicz and Biesiadka 1975	
Acilius	0.00	0.00	0.42	*semisulcatus*: this study	
Buenoa	0.00	0.00	0.63	*scimitra*: this study	
Mesostoma	0.00			*ehrenbergii*: Maly et al. 1980	
Gammarus	0.04			*lacustris*: Anderson and Raasveldt 1974	
$\bar{X}$	0.05	0.07	0.59		
SE	0.03	0.07	0.09		

ful in capturing agile prey often have rapid attack responses (e.g. odonates, chaoborids, copepods, particulate-feeding fish), whereas predators that are less successful at capturing evasive prey often have slower attacks (Pritchard 1965; Kerfoot 1977; Janssen 1978; Pastorok 1980a, 1980b; Vinyard 1980, 1982; Williamson 1983). In many cases, there appears to be a relationship between predator foraging speed and attack acceleration; i.e. cruising predators often show little acceleration when attacking prey, whereas ambush predators often have rapid strikes.

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Growth and Production of *Eogammarus confervicolus* (Amphipoda: Anisogammaridae) at a Log Storage Site and in Areas of Undisturbed Habitat within the Squamish Estuary, British Columbia

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Stanhope, M. J., and C. D. Levings. 1985. Growth and production of *Eogammarus confervicolus* (Amphipoda: Anisogammaridae) at a log storage site and in areas of undisturbed habitat within the Squamish estuary, British Columbia. Can. J. Fish. Aquat. Sci. 42: 1733–1740.

Growth and production of *Eogammarus confervicolus* was investigated in three contrasting habitats within the Squamish estuary: a log debris area, an embankment along the perimeter of a *Carex lyngbyei* marsh, and a *Fucus distichus* community. *Eogammarus confervicolus* annual production was highest in the "bank" habitat (21.38–21.65 $g \cdot m^{-2} \cdot yr^{-1}$) followed by *Fucus* (11.31–12.99 $g \cdot m^{-2} \cdot yr^{-1}$) and then wood debris (6.12–6.75 $g \cdot m^{-2} \cdot yr^{-1}$). *P*:*B* ratios were similar for the bivoltine wood debris and bank animals (4.67–5.15 and 5.94–6.01, respectively) but lower for the univoltine *Fucus* population (3.28–3.76). Growth rates were judged to be higher in bank and *Fucus* than in wood debris. Mortality rates were highest in wood debris. The food available for estuarine amphipods in wood debris habitats is thought to be inferior to that produced in marsh and algal communities. Despite the fact that *E. confervicolus* production in wood debris was much lower than in undisturbed locations, the possibility remains that such areas are still adequate food gathering areas for juvenile salmonids, if water quality conditions permit fish access to the log storage site.

Les auteurs ont étudié la croissance et la production de *Eogammarus confervicolus* dans trois habitats différents de l'estuaire de la Squamish : une zone de détritus ligneux, un talus le long du périmètre d'un marécage dominé par *Carex lyngbyei* et une communauté de *Fucus distichus*. La production annuelle de *E. confervicolus* était plus élevée sur le talus (21,38–21,65 $g \cdot m^{-2} \cdot an^{-1}$); venaient ensuite la communauté de *Fucus* (11,31–12,99 $g \cdot m^{-2} \cdot an^{-1}$), puis la zone de détritus ligneux (6,12–6,75 $g \cdot m^{-2} \cdot an^{-1}$). Les rapports *P*:*B* étaient semblables pour les animaux bivoltins peuplant les déchets ligneux et le talus (4,67–5,15 et 5,94–6,01 respectivement) mais moins élevés chez la population univoltine fréquentant la communauté de *Fucus* (3,28–3,76). On a conclu que les taux de croissance étaient plus élevés sur le talus et dans la communauté de *Fucus* que dans la zone de débris ligneux, où les taux de mortalité étaient plus élevés. On a aussi considéré que la quantité de nourriture disponible pour les amphipodes estuariens dans les habitats composés de débris ligneux était inférieure à celle produite dans le marécage et la communauté d'algues. Malgré le fait que la production de *E. confervicolus* dans la zone de débris ligneux était beaucoup moins élevée qu'aux endroits vierges, il demeure possible que de tels endroits soient des zones d'alimentation adéquates pour les jeunes salmonidés, si les conditions de la qualité de l'eau permettent aux poissons d'accéder au site de stockage.

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The forestry and fishing industries in British Columbia have important interests in estuaries. The flat land around estuaries provides better access to water and the sheltered environment affords easier log storage and handling. Estuaries are thought to be critical nursery and feeding grounds for many juvenile fish, most notably salmonids, in British Columbia waters.

The practice of log storage in estuarine intertidal areas is a major concern to fisheries. Estuarine log storage can result in compact sediment, high organic content, anoxic conditions, loss of eelgrass (*Zostera marina*) beds, destruction of marshes due to grounding logs, and the physical alteration of the benthic substrate through the settling of bark fragments. All of these consequences of log handling activities have been assumed to lead to the loss of important fish food organisms for juvenile salmonids.

Despite negative assumptions about logging effects and the apparent conflict of interest between forestry and fishing, there have been only a few investigations designed to determine the effects of log handling practices on benthic organisms (e.g. see Conlan and Ellis 1979; Sibert and Harpham 1979; Levy et al. 1982; McGreer et al. 1984).

The accumulated evidence points to a general reduction of total benthic invertebrate density and diversity in areas of log

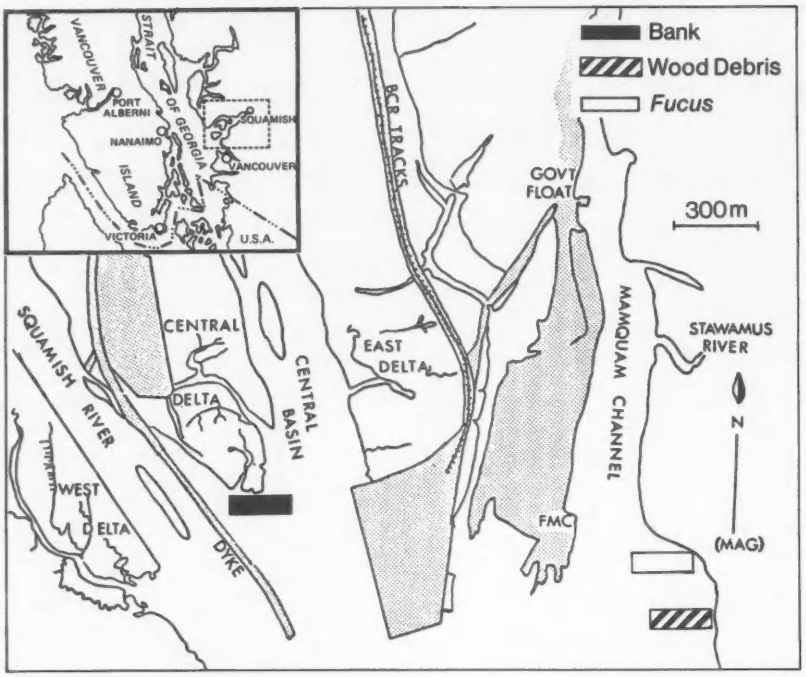

FIG. 1. Squamish River estuary showing the major physical features and sampling locations (from Levy and Levings 1978). Stippled areas indicate land fills.

storage. Similar negative effects on estuarine benthic invertebrates important as salmon food have not been clearly demonstrated. The few investigations to date have considered animal abundance but not detailed aspects of population biology. Secondary production is one such population characteristic that may be useful in assessing the effects of man-made disturbance. The purpose of this study was to examine the effects of log storage activities, specifically the accumulation of bark fragments, on the growth and production of *Eogammarus confervicolus* in the Squamish estuary, British Columbia. *Eogammarus confervicolus* is the most abundant and widely distributed gammarid amphipod on the north Pacific coast (Bousfield 1979). It is a major food organism for juvenile salmonids in the Squamish and other estuaries, as well as comprising a portion of the diet in herring, sculpin, and flounder (Goodman and Vroom 1972; Levy and Levings 1978; Levy et al. 1982).

Study Area

Logs have been stored on the eastern side of the Squamish estuary (Fig. 1) for approximately 50 yr. The result has been the accumulation of bark fragments in depressions along the Mamquam intertidal mud flat. *Eogammarus confervicolus* was found within these bark fragments. Bark pieces ranged in size from very fine particles less than 250 μm to pieces several metres long and were layered between a few centimetres and 25 cm deep. Sand (median grain size 300 μm) was present beneath the wood debris as well as in interstitial spaces between bark fragments. Remnant patches of vegetation suggest that this mud flat was once a marsh.

The Mamquam intertidal flats have a slope of about 1% and extend 500 m from the high to low tide marks. At about 3.5 m above chart datum the wood debris gives way to a zone of *Fucus distichus* and *Pelvetia fastigiata* (hereinafter termed *Fucus*) overlying soft dark black mud smelling of hydrogen sulphide. The amphipods were found within the algal mixture and at the mud surface.

On the western side of the estuary at the central delta there has been only limited log storage. The delta is a *Carex lyngbyei* marsh community, the outer edge of which is an embankment. Hanging over this bank is a mat composed of sand, clay, and *C. lyngbyei* rhizomes, in which *E. confervicolus* was found. These areas represent three of the major intertidal habitat types for *E. confervicolus* in the Squamish estuary.

Salinity ranged from as low as 2‰ in June, to 24‰ in February. Temperature fluctuated between 4°C in January to 18°C in July. Salinity and temperature on any given date were similar across habitats.

Materials and Methods

Quadrat (0.06 m^2) collections were taken at monthly intervals between June 1981 and September 1982 in each of the areas. Eight quadrats were taken from each location on each date. On the Mamquam delta a sampling plot 10 × 10 m was established in a typical wood debris area at 2.0 m above chart datum. A similar plot was established in the *Fucus*. All the material contained within randomly placed quadrats was collected to a depth 2–5 cm below sediment surface. At the central delta, collections were made by placing the quadrat against the

rhizome mat and cutting out all the material contained within. All the animals and substrate greater than 500 μm were retained and preserved in 10% formalin with Rose Bengal. The amphipods were separated from the substrate by floating the samples in a salt solution. This procedure was successful in separating over 95% of the animals from the accompanying debris. Animals were counted and sized using a stereomicroscope or by comparing a specimen with sets of previously measured individuals (Hynes 1955). Measurements of *E. confervicolus* head lengths were converted to total length using the relationship determined by Levings (1980): TL = 9.63HL − 2.09. Animals were separated into 1.0-mm size classes. Dry weights of *E. confervicolus* were determined using the following length–weight relationship: log (dry weight) = 3.4783 log (HL) + 0.2125 (Pomeroy and Levings 1980).

Eogammarus confervicolus productivity was calculated using the method of Hynes and Coleman (1968), as corrected by Hamilton (1969) and Benke (1979), and by the cohort summation of losses method (Crisp 1971). Cohorts were separated using Harding (1949) probability plots.

Growth of *E. confervicolus* from newly released juveniles to adults was followed in simulated natural conditions. A series of outside aquaria was established at the Department of Fisheries and Oceans West Vancouver Laboratory. Substrate from each of the estuarine wood debris, "bank," and *Fucus* habitats was brought back to the laboratory and amphipods removed. Four aquaria for each substrate type were established. Each aquarium (200 L) was inoculated with 1500–2000 juvenile *E. confervicolus*. Animals were placed in the experimental substrate from which they were collected. The experiment commenced in early June 1982 and terminated in October 1982. Salinity of the static water was maintained the same as that of the estuary. This ranged between 3 and 8‰ in June, July, and August and between 8 and 13‰ in September and October. Tide was simulated by controlling a drain in each aquarium that was surrounded by fine-mesh screen to prevent the loss of either substrate or animals. One tidal cycle per day was established, with low tide from 10:00 to 16:00 and high tide during the evening, night, and early morning. Every week, 25 *E. confervicolus* were sampled from each of two aquaria. Two aquaria of a particular substrate were sampled on even weeks and the other two on odd weeks. The aquarium pairs were compared every week using an analysis of variance and were found not to be significantly different for any date and were thus pooled. Odd and even weeks data were then combined in the construction of growth curves.

Density and biomass estimates were analyzed using an analysis of variance followed by a Duncan's multiple range test. Growth and mortality data were analyzed using an analysis of covariance. A slope test on survivorship data was used to distinguish mortality rates among locations (H_0:$\beta_1 = \beta_2 = \beta_3$ and if rejected: H_0:$\beta_1 = \beta_2$; H_0:$\beta_1 = \beta_3$; H_0:$\beta_2 = \beta_3$). Analysis of cohort growth followed the same procedure. Analysis of growth in the laboratory conditions was according to the following: H_0:$\beta_1 = \beta_2 = \beta_3$ and if accepted a Duncan's multiple range test ranked the adjusted means; if rejected the same procedure described for mortality and cohort growth was followed. The analysis of mortality rates and cohort growth only considered slope, since neither the initial density or size was the same in the three situations, whereas in the laboratory growth situation all animals at the commencement of the experiment were the same size. Null hypotheses were rejected at $P < 0.05$.

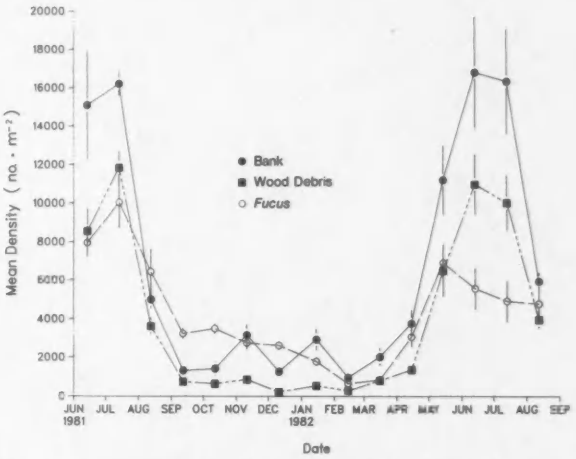

FIG. 2. Mean monthly densities (with standard errors) of *E. confervicolus* in each of the respective habitat types.

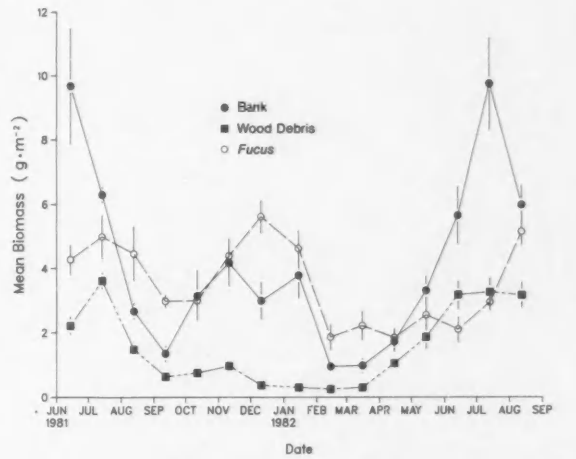

FIG. 3. Mean monthly biomass (with standard errors) of *E. confervicolus* in each of the respective habitat types.

Results

Mean monthly densities of *E. confervicolus* (Fig. 2) differed with regard to habitat and sampling times, and there was a significant interaction between habitat and date. All the mean annual densities (bank: $6233 \cdot m^{-2}$; wood debris: $3603 \cdot m^{-2}$; *Fucus*: $4241 \cdot m^{-2}$) were significantly different. The results suggest that although the general trend for all three habitats was one of high abundance in summer months followed by a decline in winter, the seasonal effects manifested themselves differently in each of the areas.

Biomass data for the wood debris and bank areas exhibited a similar trend: high biomass in summer months, followed by a decline in the fall, with a smaller peak in the late fall and midwinter (Fig. 3). The *Fucus* site supported a more consistent level of biomass in the summer, fall, and winter months, followed by a decline in the spring. The wood debris consis-

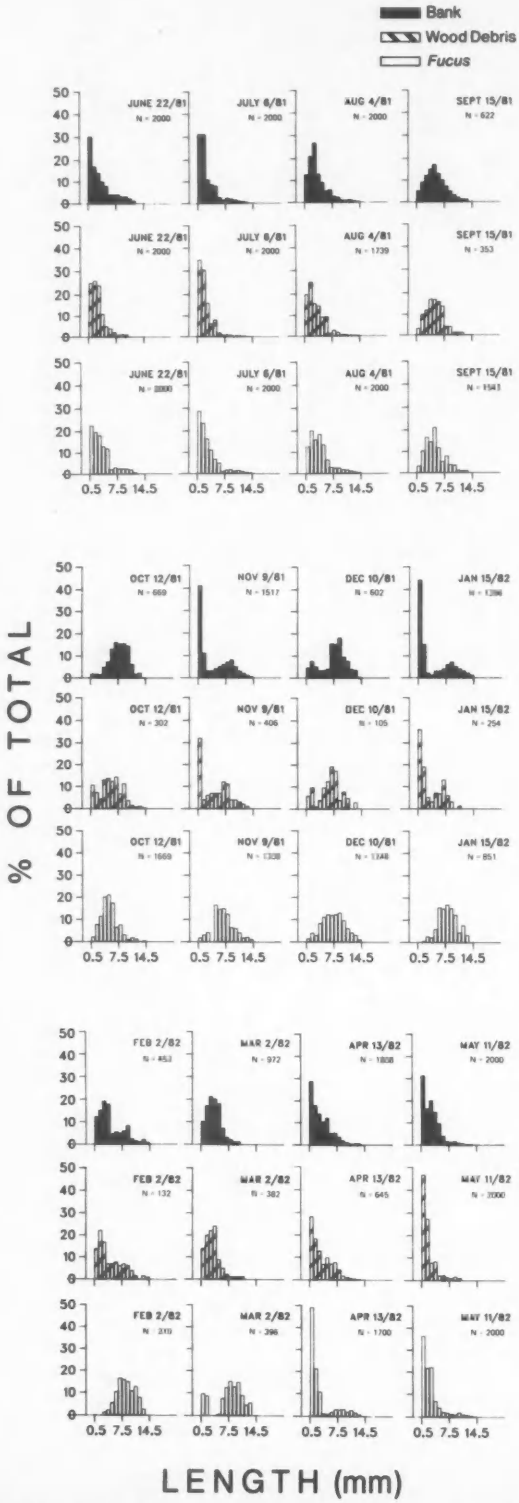

FIG. 4. Size frequency data of *E. confervicolus* from each location.

tently supported lower biomass than either the *Fucus* or bank habitats. Biomass differed with regard to habitat and date, and there was a significant interaction between habitat and sampling time. Mean annual biomasses of the bank and *Fucus* locations (3.60 and 3.45 $g \cdot m^{-2}$, respectively) were indistinguishable, and the wood debris value (1.31 $g \cdot m^{-2}$) was significantly different from the bank or *Fucus* means.

The size frequency distribution within each habitat (Fig. 4) helps explain the observed trends in density and biomass. The distributions from the wood debris and bank areas were similar; most of the population consisted of juveniles in June 1981, which attained midsize by fall, and were responsible for a reproductive pulse in November when a small increase in numbers and biomass therefore occurred. The increases in the wood debris were much less marked because of the lower population densities. Reproduction continued throughout the winter; by spring the animals produced in winter had almost reached midsize, and by April another pulse of juveniles appeared. These data indicate two generations per year, with overlapping cohorts. The *Fucus* area displayed a similar trend up to November but did not show the winter reproductive period. The adult mortality was much less pronounced than in the wood debris and bank sites; thus, the biomass remained higher. Adult mortality in late winter lowered the biomass, and spring reproduction was not sufficient to raise it to the levels of the preceding summer. Cohort production intervals (Benke 1979) were estimated from size frequency data at 411 d for *Fucus*, 279 d for bank, and 336 d for wood debris.

Growth of individuals in the estuary (Fig. 5A) was significantly faster in bank substrate, followed by *Fucus* and then wood debris. Growth of winter and summer cohorts (bank and wood debris) was not significantly different. Growth of individuals under laboratory conditions (Fig. 5B) was significantly faster in *Fucus*, followed by bank and then wood debris. Mortality rate of the summer cohort (Fig. 6A) was greatest in wood debris, followed by bank and then *Fucus*; winter cohorts (Fig. 6B) were not significantly different. The fluctuating survivorship curves for the winter cohort reflect the very high mortality of newly released juveniles during the November to February period. After February, however, mortality rate of the wood debris winter cohort was significantly reduced from its summer counterpart.

Annual production (Table 1) was highest in bank (21.38–21.65 $g \cdot m^{-2} \cdot yr^{-1}$), more than three times that of the wood debris area (6.12–6.75 $g \cdot m^{-2} \cdot yr^{-1}$). *Eogammarus confervicolus* production in *Fucus* was about double that of the wood debris value. The two methods of calculation resulted in very similar estimates. Annual *P:B* ratios (Table 1) were comparable in the wood debris and bank locations and considerably lower in the *Fucus* area. Summer cohorts were considerably more productive, especially in wood debris, than their winter counterparts. Cohort *P:B*'s were identical in the bank habitat but quite disparate in wood debris, likely due to the much increased mortality of the summer versus winter cohort (see Allen 1971 for theoretical discussion of factors affecting *P:B* ratios). Summer productivity values for 1981 and 1982 were almost identical in the bank habitat but considerably different in wood debris and *Fucus*.

Discussion

The high densities reported here for the bank habitat are comparable with those reported by Levings (1980) for the same

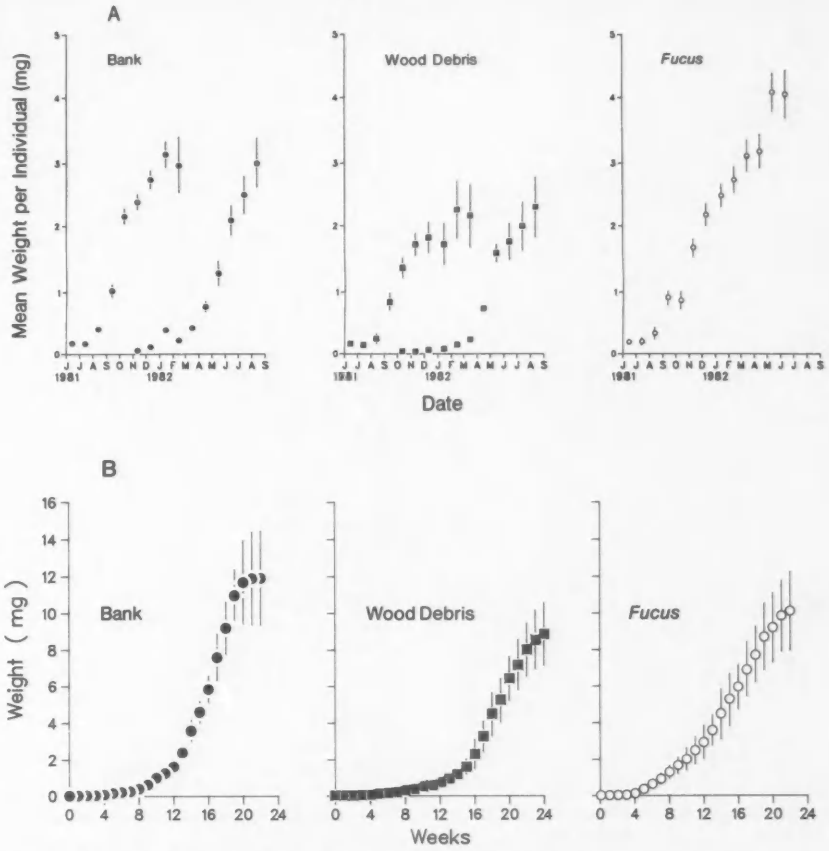

FIG. 5. Growth of *E. confervicolus* (with 95% confidence limits) in (A) estuary and (B) laboratory habitats.

area in the summer of 1973 (maximum $14\,000 \cdot m^{-2}$). The mean annual density over that period was $4108 \cdot m^{-2}$. Levings did not sample the Mamquam wood storage area but did sample a section of the western delta where there was wood debris. Mean annual density was $2020 \cdot m^{-2}$ with a peak abundance in May of $5450 \cdot m^{-2}$. Densities of gammarid amphipods comparable with those found in the sedge mat are rare in the literature. Population density estimates of marine or estuarine gammarids usually are not within the same order of magnitude as the maxima observed in any of the three areas (e.g. see Stoner 1980; Hughes 1982; Levy et al. 1982; Schaffner and Boesch 1982; Edgar 1983; McGreer et al. 1984). Levings (1980) suggested that a training wall built along the Squamish River (see Fig. 1) has minimized flushing of the central delta and thus resulted in the high densities present in the sedge mat.

Eogammarus confervicolus productivity at the central delta was much greater than most amphipod productivity estimates reported elsewhere. Only those reported by Tilly (1968; $36.6\,g \cdot m^{-2} \cdot yr^{-1}$) and Waters and Hokenstrom (1980; $27.1\,g \cdot m^{-2} \cdot yr^{-1}$) for *Gammarus pseudolimnaeus* exceed our bank estimates. In Waters' (1977) comprehensive review of production estimates of aquatic invertebrates, only 2 of 118 (in addition to Tilly's) exceed our central delta figure and only 16 are greater than $6.0\,g \cdot m^{-2} \cdot yr^{-1}$. *Eogammarus confervicolus* productivity at the central delta, then, was exceptionally high, as was the level of production sustained in the *Fucus* area. The wood debris estimate, although less than one third that of the bank habitat, is larger than most aquatic invertebrate production values.

The lower productivity of *E. confervicolus* in the Squamish wood debris habitat, relative to the undisturbed locations, appears not to be peculiar to this epibenthic organism. Stanhope (1983) found the annual production of *Corophium spinicorne* (Amphipoda: Corophiidae) in the wood debris area to be $0.78\,g \cdot m^{-2} \cdot yr^{-1}$ as opposed to $4.66\,g \cdot m^{-} \cdot yr^{-1}$ in *Fucus*. Productivity of mud patches devoid of wood chips that were immediately adjacent to areas of wood debris was estimated (from *P*:*B* ratio) to be about $12\,g \cdot m^{-2} \cdot yr^{-1}$. Secondary production of *Gnorimosphaeroma oregonensis* (Isopoda: Sphaeromatidae) was 26.77, 3.87, and $13.46\,g \cdot m^{-2} \cdot yr^{-1}$ for the bank, wood debris, and *Fucus* areas, respectively (M. J. Stanhope and D. Powell, unpubl. data). There is some indication, then, that wood debris is an inferior estuarine habitat for epibenthic organisms.

Most of this productivity occurred in summer, especially in bank and wood debris locations (see Table 1). This coincides with the peak periods of chum (*Oncorhynchus keta*), chinook (*O. tshawytscha*), and coho (*O. kisutch*) salmon fry abundance in Squamish, May–July (Levy and Levings 1978). Chum fry are the most abundant salmonid in the estuary. We computed chum density using data from Goodman and Vroom (1972),

TABLE 1. Summary of production data.

Habitat	Annual P: Cohort summation of losses ($g \cdot m^{-2} \cdot yr^{-1}$)	Annual P: Size frequency ($g \cdot m^{-2} \cdot yr^{-1}$)	Annual *P:B*	Cohort P: Summer cohort ($g \cdot m^{-2}$)	Cohort P: Winter cohort ($g \cdot m^{-2}$)	Cohort *P:B*: Summer cohort	Cohort *P:B*: Winter cohort	Summer P (June–September): 1981 ($g \cdot m^{-2}$)	Summer P (June–September): 1982 ($g \cdot m^{-2}$)
Bank	21.65	21.38	5.94–6.01	9.88	5.08	4.15	4.14	15.58	15.49
Wood debris	6.12	6.75	4.67–5.15	4.32	1.10	5.96	2.95	4.53	6.22
Fucus	12.99	11.31	3.28–3.76	—	—	—	—	4.81	3.79

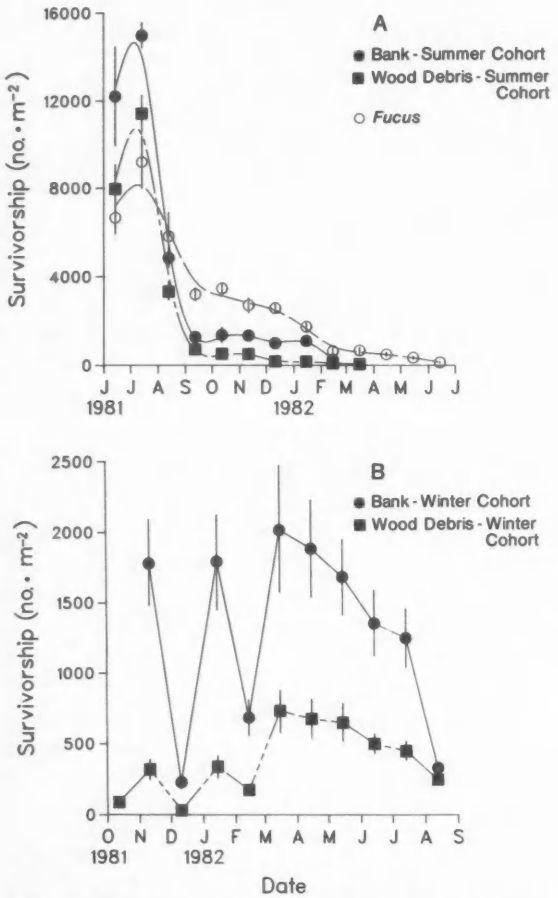

FIG. 6. Survivorship curves (with standard errors) for (A) summer and (B) winter cohorts.

Levy and Levings (1978), and P. J. Ryall (Department of Biological Sciences, Simon Fraser University, Burnaby, B.C. V5A 1S6, unpubl. data for 1985). At the time of the maximum chum catches in each of the 3 yr, mean fry density was approximately 0.2 fish $\cdot m^{-2}$ in the central delta channel, near the bank habitat examined in the present study; Goodman and Vroom (1972) found fish numbers in the wood debris area to be much less, which they suggested may be due to poor water quality. A value of 0.2 fish $\cdot m^{-2}$ is similar to that obtained in the Fraser estuary marshes (Levy and Northcote 1982) and the intertidal mud flats of the Nanaimo estuary (Healey 1979). According to Healey, a chum population of this density represents an average daily food requirement of 0.01 $g \cdot m^{-2}$ (wet weight). Since productivity in the bank habitat, between April 15 and June 22 (period of peak chum abundance), was 0.43 $g \cdot m^{-2} \cdot d^{-1}$ (wet weight) and at the wood debris site 0.09 $g \cdot m^{-2} \cdot d^{-1}$ (wet weight), food was presumably not limiting chum production in either location. At a consumption rate of 15% body weight per day (Healey 1979), all *E. confervicolus* production would be consumed at a chum density of 5.2 $\cdot m^{-2}$ in the central delta channel and at a density of 2.8 $\cdot m^{-2}$ at the Mamquam wood debris area (estimated by using planimetry to determine area of each habitat type, from which total food produced was determined, using a fish residence time of 10 d and a mean size of 0.66 g). Maximum coho and chinook densities in the Squamish estuary are approximately 0.08 and 0.1 $\cdot m^{-2}$, respectively (Levy and Levings 1978), and the runs only overlap slightly; thus, food for salmonids would not appear to be limiting in either location. This supposition becomes less reliable when one considers that the other dominant fish in the estuary, staghorn sculpin (*Leptocottus armatus*), is present at the central delta in high numbers (densities from Squamish not available, but estimated at 1.0 $\cdot m^{-2}$ from a similar habitat in the Fraser estuary (Gordon and Levings 1984)) and is known to be a heavy consumer of *E. confervicolus* (Mace 1983). A shift in the preferred diet of the salmonids from *E. confervicolus* to *C. spinicorne* or *G. oregonensis* may be necessary during periods of intense consumption. Factors other than the level of food production, such as the role of wood chips, sedge mats, and *Fucus* as refuges and the different behavior patterns of the amphipods in the various substrates, will influence the availability of the gammarids to the salmon and may be important variables affecting the juvenile salmon. Fish may abandon the wood debris habitat when foraging success drops to a certain level, increasing the pressure on other habitats.

An explanation for the lower productivity and growth in the wood debris area may lie in the food available in each of the respective locations. Microorganisms are thought to be an important component of amphipod diets, affecting both survivorship and growth rates (Hargrave 1970; Kostalos and Seymour 1976; Sutcliffe et al. 1981). McKeag (1983) gave evidence indicating that wood chips are a relatively poor substrate for bacterial growth. She determined bacterial densities, supported from various organic substrates, under varying inorganic nutrient regimes. The substrates included two algal species, *Ulva lactuca* and *Fucus vesiculosus*, the vascular plant *Z. marina*, and wood chips. Wood chips supported bacterial densities one to three orders of magnitude less than the algae and one to two orders of magnitude less than *Z. marina*. The addition of inorganic nutrients greatly increased bacterial densities supported by the seaweeds but had no effect on bacterial

biomass supported by wood chips. Preliminary results of our own, obtained by sonicating small wood chips and *Fucus* fragments and observing the resulting suspended material using epifluorescent microscopy, support McKeag's findings. Estuarine wood chips supported much lower bacterial, diatomaceous, and fungal biomasses than did *Fucus* blades.

A question that becomes germane is "to what degree can these results be generalized to other estuaries in which logs are stored?" The idea of estuarine comparisons has been given some attention in recent years (e.g. see Kennedy 1982). It is an issue of critical importance to the future of estuaries, but as yet insufficient documentation of similarities and differences on both local and worldwide scales exists to reliably extrapolate existing information. All three habitat types examined in this study are common in estuaries of Alaska, British Columbia, Washington, and Oregon. If one applies the *P:B* ratios to biomass data collected at the central delta in 1972–73 (Levings 1973), there has been little decrease in production in 10 yr (approximately $27\ g \cdot m^{-2} \cdot yr^{-1}$ during 1972–73). Summer productivity in 1981 and 1982 was almost identical in the bank habitat. *Fucus* and wood debris habitats are apparently less stable environments; the 1982 summer productivity in wood debris was $1.69\ g \cdot m^{-2}$ greater than the productivity of the previous summer. Lindeman and Momot (1983) calculated secondary production of *Hyalella azteca* in three habitat types within a lake in northern Ontario over two consecutive years. Productivity for a given area was similar from year to year and the habitat ranking was identical (interestingly, their most productive habitat was a stand of *Carex aquatilis*). Particular habitat types may be capable of supporting particular levels of amphipod production. Data from other estuaries with similar and additional habitat types could form the basis of a useful management tool for assessing the potential fisheries productivity of a given area.

Despite the fact that amphipod production in wood debris was lower than in undisturbed locations, it remains, compared with other data in the literature, a relatively high value. This is undoubtedly a consequence of the highly productive nature of estuaries as compared with subtidal areas, lakes, and streams. We would like to point out that although our results indicate that log debris areas are an inferior estuarine amphipod habitat, estimates of amphipod consumption by Squamish salmonids suggest the possibility that such areas are adequate food gathering areas for juvenile salmonids. This in turn accentuates the need for experiments designed to determine the availability of benthic food organisms to juvenile salmonids in various substrate types.

Acknowledgements

This work was made possible through the assistance afforded M. Stanhope during his graduate research by MacMillan Bloedel Limited. Special thanks are due to W. W. Bourgeois, P. G. Bruce, and particularly W. R. Pollard of the company's Land Use Planning Advisory Team for their kind support. L. Pomeroy and J. Beattie assisted in the collection and processing of samples. Dr. N. Sloan offered constructive advice during the early stages of the project.

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Aluminum Bioaccumulation and Toxicity to *Daphnia magna* in Soft Water at Low pH

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Havas, M. 1985. Aluminum bioaccumulation and toxicity to *Daphnia magna* in soft water at low pH. Can. J. Fish. Aquat. Sci. 42: 1741–1748.

Aluminum may be either harmful or beneficial to *Daphnia magna* (Straus) depending on pH and on the Al concentration in the water. My results are based on laboratory experiments conducted at various concentrations of total Al (0.02–1.02 mg/L) in soft water (2.5 and 12.5 mg Ca/L) adjusted from pH 6.5 to 4.5. Maximum Al toxicity and maximum Al bioaccumulation were observed at pH 6.5 (at and above 0.32 mg total Al/L). At lower pHs (≤5.0), H^+ was toxic to *D. magna*. Aluminum (1.02 mg/L) temporarily ameliorated H^+ toxicity at pH 4.5. Calcium reduced H^+ toxicity at pH 5.0 and Al toxicity at pH 6.5. Mortality in the presence of Al and also at low pH was associated with a net loss of Na and Cl from the daphnids. The Ca content of the daphnids was highly variable and showed no consistent pattern apart from a negative correlation with the Al content of the daphnids at pH 5.0 and 5.5. The 24-h bioconcentration ratio for Al was 10 000 at pH 6.5, 4000 at pH 5.0, and negligible at pH 4.5. The rapid uptake of Al, particularly at circumneutral pHs, may be an additional source of Al for zooplanktivorous fish and other predators.

L'aluminium peut être soit nuisible ou salutaire à *Daphnia magna* (Straus) selon le pH et la concentration aqueuse de Al. Ces résultats sont tirés d'expériences en laboratoire menées à diverses concentrations de Al total (de 0,02 à 1,02 mg/L) dissous en eau douce (de 2,5 à 12,5 mg/L de Ca) dont le pH a été porté de 6,5 à 4,5. La toxicité et la bioaccumulation maximales de Al ont été observées au pH 6,5 (à une concentration de 0,32 mg/L et plus de Al total). À des pH inférieurs (≤5,0), le H^+ était toxique pour *D. magna*. Au pH 4,5, l'aluminium (1,02 mg/L) a temporairement amélioré la toxicité de H^+ tandis que le calcium l'a réduite au pH 5,0. Par contre, le calcium a réduit la toxicité de Al au pH 6,5. La mortalité en présence de Al à de faibles pH était associée à une perte nette de Na et de Cl chez les daphnies dont la teneur en Ca variait beaucoup et ne montrait aucun régime uniforme à part une corrélation négative avec la teneur en Al des daphnies aux pH 5,0 et 5,5. Aux pH 6,5 et 5,0, le rapport de la bioconcentration de Al après 24 h s'élevait respectivement à 10 000 et à 4000; il était négligeable au pH 4,5. La captation rapide de Al, surtout à des pH presque neutres, peut représenter une source supplémentaire de Al pour les poissons zooplanctivores et d'autres prédateurs.

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The importance of aluminum (Al) in acidic lakes has only recently been recognized by aquatic scientists although geochemists, agronomists, and plant ecologists have been increasingly aware of the complex chemistry of Al and of its toxicity to plants growing in acidic soils since the early 1900s (Hartwell and Pember 1918; Magistad 1926). Concentrations of Al are high in soils, but most of this Al is tightly bound. However, when acidic water comes in contact with soil the Al becomes more soluble and more available to plants and animals.

Leaching of Al into lakes and streams appears to have accelerated in regions exposed to acidic precipitation (Dickson 1980; Wright et al. 1980). Whereas concentrations of Al in circumneutral, oligotrophic lakes seldom exceed 0.05 mg/L, in similar acidic lakes, concentrations in excess of 0.2 mg/L are becoming increasingly common (Havas 1985).

The loss of fish populations in parts of North America and Scandinavia has been attributed, in part, to elevated Al concentrations (Grahn 1980; Schofield and Trojnar 1980; Bengtsson et al. 1980). Laboratory experiments have confirmed that concentrations of Al found in many of these lakes are toxic to fishes (Baker 1982; Brown and Lynam 1982; van Coillie et al. 1983). Toxicity is associated with respiratory stress and problems with salt regulation (Muniz and Leivestad 1980).

Little is known about the effects of Al on aquatic invertebrates. Changes in species composition, which occur as lakes acidify, cannot readily be attributed to any one factor. Both low pH and low concentrations of Ca can limit the distribution of aquatic invertebrates (Okland 1980), but complex biotic interactions (Eriksson et al. 1980) and increased concentrations of metals, such as Al (Havas and Hutchinson 1982), are also likely to be important.

My purpose was to determine the effects of pH and Ca concentrations on Al toxicity to the water flea, *Daphnia magna* (Straus). I also examined the physiological basis of Al toxicity as it relates to Na, Cl, and Ca regulation and to Al bioaccumulation.

Materials and Methods

Daphnia magna, obtained from the Department of Natural Resources, Cornell University, was cultured and tested in water collected in June 1982 from Mirror Lake, New Hampshire (pH

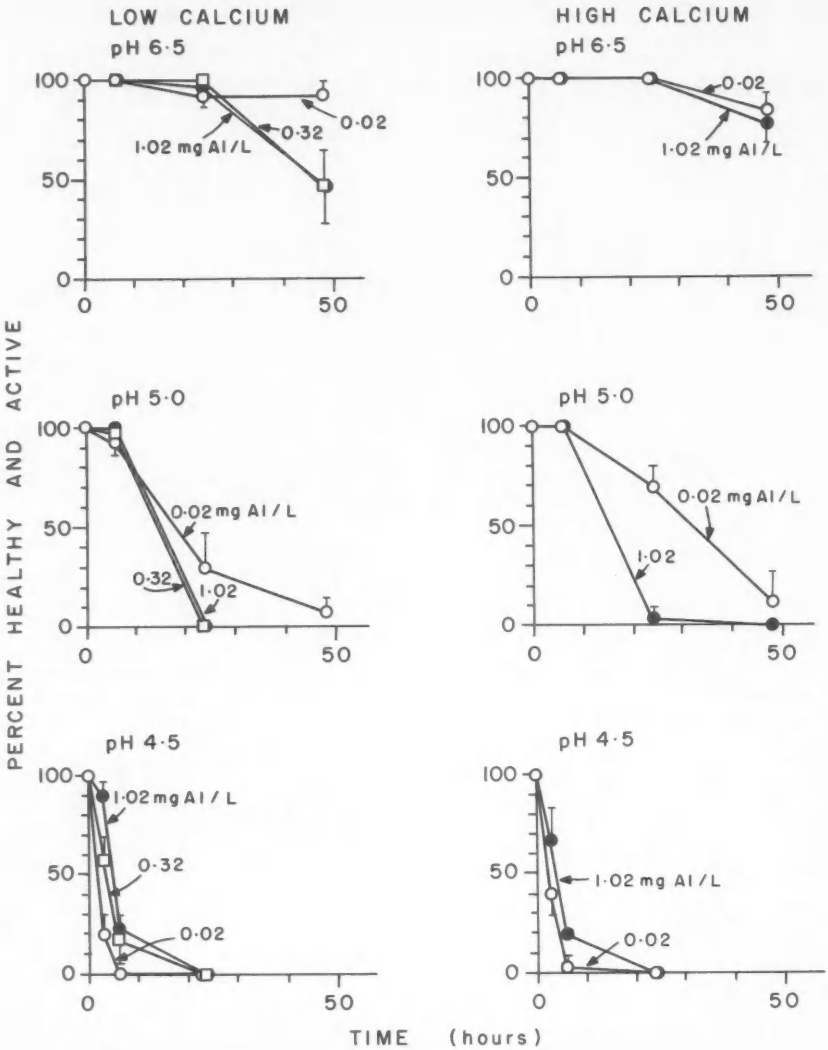

FIG. 1. Survival of *D. magna* exposed to low pH (6.5, 5.0, 4.5) and elevated concentrations of Al (0.02 reference, 0.32, 1.02 mg/L) in water containing 2.5 mg Ca/L (low Ca) and 12.5 mg Ca/L (high Ca). Vertical bars represent 1 SD of three replicate samples.

6.6, 2.45 mg Ca/L, 0.52 mg Mg/L, 1.76 mg Na/L, 0.49 mg K/L, 2.07 mg Cl/L, 5.3 mg SO_4/L, 0.02 mg Al/L, see Havas and Likens 1985a). *Daphnia* were fed *Scenedesmus* daily and brewers' yeast on alternate days. Cultures were maintained at room temperature (18–20°C) with a 14 h light : 10 h dark photoperiod. Only mature daphnids between 2 and 3 mm in length were used.

Mirror Lake water was filtered through a 70-μm-pore sieve to remove large zooplankton and was then stored at room temperature for 2 wk, when it was aerated and sieved once more. The pHs were adjusted using Analar grade 0.005 mol H_2SO_4/L and 0.01 mol NaOH/L to pH 4.5 (range 4.4–4.7), 5.0 (range 5.0–5.8), and 6.5 (range 6.4–6.8, ambient or reference pH). These were checked and readjusted after 48 h to ensure CO_2/air equilibration and were readjusted after 6, 12, and 48 h during the experiment.

A stock solution of 100 mg Al/L was prepared by dissolving $AlCl_3 \cdot 6H_2O$ crystals in distilled, deionized water to which Analar grade HNO_3 was added to give a final pH not greater than 3. At this pH, all of the Al remained in solution as free Al^{3+}. Concentrations of total Al thus ranged from 0.02 (ambient) to 1.02 mg/L. Calcium was added as $CaCl_2$ to increase the concentration from 2.5 (ambient) to 12.5 mg/L.

To determine the effects of Al (0.02, 0.32, and 1.02 mg/L), pH (6.5, 5.0, and 4.5), and Ca (2.5 and 12.5 mg/L) on survival of *D. magna*, I conducted experiments in 50-mL plastic vials containing 30 mL of Mirror Lake water and 10 daphnids. The percentage of "active" (healthy, able to swim and filter-feed normally), "inactive" (dying, unable to swim or filter-feed), and "dead" (no response to tactile stimulus, no obvious heartbeat) individuals was recorded for up to 48 h. Results are based on three replicate samples each consisting of 10 daphnids. *Daph-*

nia were not fed during the experiment but were maintained under the culture conditions previously described.

To determine the effects of Al, pH, and Ca on the chemical composition of *D. magna*, I used a setup identical to the first experiment. After 0, 3, 6, 24, and 48 h the daphnids were removed from the vials, rinsed with deionized water, oven-dried at 60°C for 48 h, and analyzed for Na, Cl, Ca, and Al content. The active, inactive, and dead daphnids were recorded but were not separated prior to chemical analysis. Results were based on three replicate samples each consisting of 10 individuals.

To compare the chemical composition of individuals that were active (healthy), inactive (dying), or dead following exposure to Al, 300 daphnids were placed into approximately 500 mL of Mirror Lake water containing 1.02 mg Al/L at pH 5.5 ± 0.4. After 24 h the *Daphnia* were placed into one of the three categories mentioned above. They were then harvested for chemical analysis as in the second experiment. Results are based on three replicate samples each containing 20 individuals. Remaining daphnids were discarded.

Total Na, Cl, Ca, and Al content of the daphnids was determined by Instrumental Neutron Activation Analysis (INAA) using the TRIGA Reactor, Cornell University. Samples were irradiated for 300 s at 10^{12} neutrons·cm^{-2}·s^{-1}. They were immediately transferred to nonirradiated vials, weighed, and counted for 300 s after a 4.0-min delay on an ENGN ORTEC high-purity Ge detector. The following isotopes and energies were used: ^{24}Na at 1368.4 keV, ^{38}Cl at 2166.8 keV, ^{49}Ca at 3083.0 keV, and ^{28}Al at 1778.9 keV. Of these, only Al has some interference from phosphorus (^{31}P) which is converted into and has the same decay characteristics as ^{28}Al.

The degree of interference from ^{31}P was calculated by irradiating a known amount of P determining the resulting ^{28}Al concentration, comparing this value with the P content of *D. magna*, which was analyzed using Inductively Coupled Plasma Emission (ICP) at the University of Toronto after a nitric acid digestion. Aluminum is approximately 1000 times more readily activated than P by INAA. Interference, therefore, only arises when the P to Al ratio in the sample is high. The calculations for *D. magna* indicate that an average P content of 17 000 μg/g should give a false Al concentration of 15 μg/g. Thus, ^{28}Al concentrations near 15 μg/g were assumed to be largely due to P. For samples containing in excess of 200 μg Al/g, I assumed that interference from P was marginal (less than 8%).

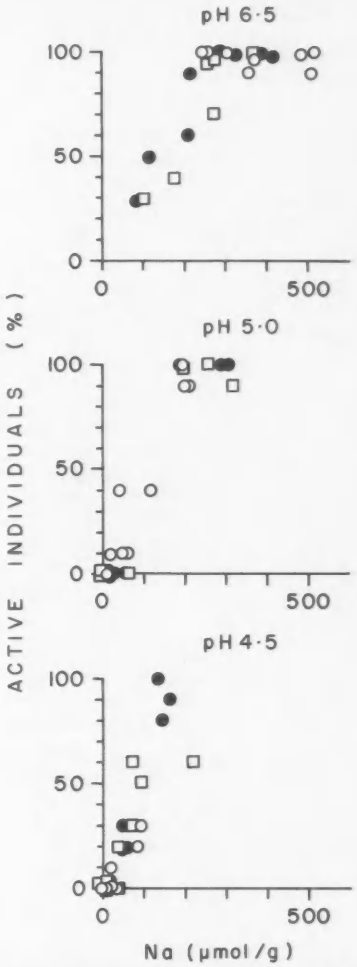

FIG. 2. Relationship between the Na content of *D. magna* and their level of activity as a function of pH and Al concentrations in the water: ○, 0.02 mg Al/L (reference); □, 0.32 mg Al/L; ●, 1.02 mg Al/L. Each point represents 10 daphnids and corresponds to the data in Fig. 1. Individuals were exposed to pH 6.5 and 5.0 for 0, 6, 24, or 48 h and to pH 4.5 for 0, 3, 6, or 24 h.

Results

Activity of *Daphnia*

Aluminum may be either harmful or temporarily beneficial depending on its concentration and on the pH of the external solution. Maximum Al toxicity was observed at pH 6.5 at ambient concentrations of Ca (2.5 mg/L) in the water. At this pH, both the 0.32 and the 1.02 mg Al/L treatments were equally toxic (Fig. 1).

A pH of 5.0 was toxic to *D. magna* in soft water. Within 24 h, more than 50% of the individuals were no longer active; many of these were dead in the low-Ca treatment (Fig. 1). Aluminum marginally increased toxicity of the water at pH 5.0.

At pH 4.5, high concentrations of Al significantly ($P \leq 0.05$, two-sample Kolmogorov–Smirnov test) reduced H^+ toxicity. Within 3 h, only 20% of the individuals were actively swimming and filter-feeding at ambient Al concentrations (0.02 mg/L) compared with 55 and 90% at 0.32 and 1.02 mg/L, respectively (Fig. 1). This amelioration, however, was short-lived, and all of the *Daphnia* died within 24 h.

At higher Ca concentrations (12.5 mg/L) there was a slight, but statistically insignificant, reduction of Al toxicity at pH 6.5 and a significant ($P \leq 0.05$, two-sample Kolmogorov–Smirnov test) reduction of H^+ toxicity at pH 5.0 (Fig. 1).

Chemical Composition of *Daphnia*

The concentration of Na in *Daphnia* decreased substantially both at low pH (≤5.0) and at toxic Al (≥0.32 mg/L) concentrations (Fig. 2). The net loss of Na was accompanied by a net loss of Cl in all of the treatments. There was a positive relationship between total body Na (or Cl) content and the percentage of active individuals. Changes in activity (i.e. swimming and filter-feeding) were detectable once the total body Na (or Cl) concentrations dropped below 250 μmol/g (5700 μg/g) at pH 6.5, 200

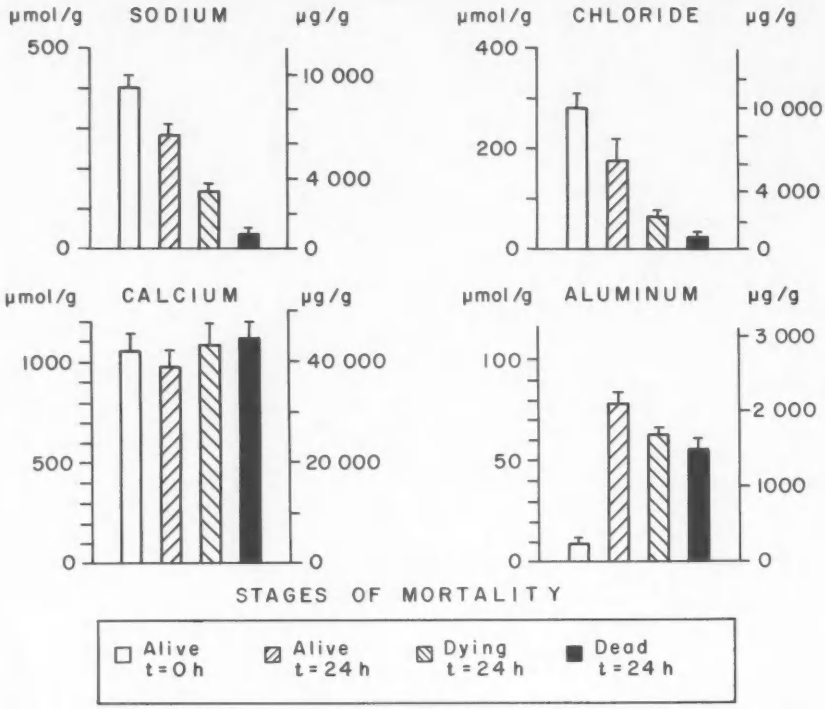

FIG. 3. Na, Cl, Ca, and Al content of *D. magna* exposed to pH 5.5 and 1.02 mg Al/L for 24 h. Individuals were grouped according to their level of activity. Vertical bars represent 1 SD of three replicate samples each containing 20 adult daphnids.

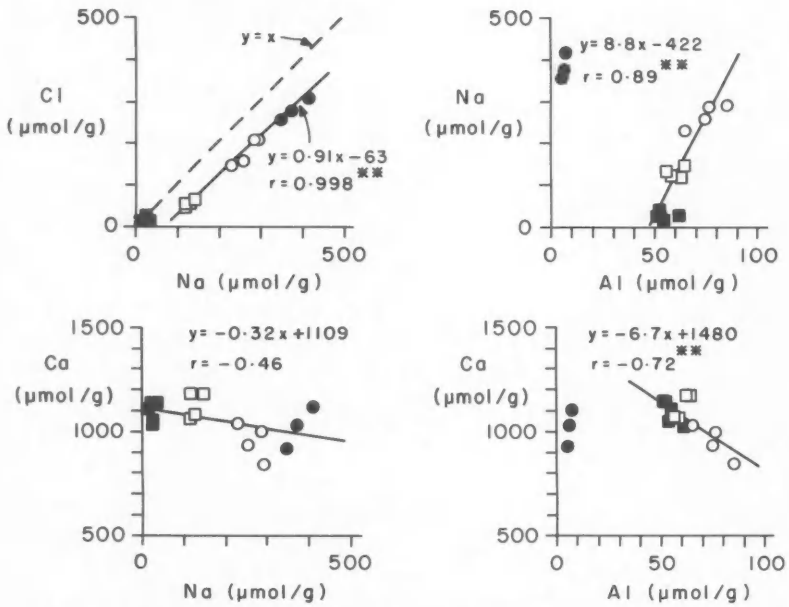

FIG. 4. Relationship between Na, Cl, Ca, and Al content of *D. magna* exposed to pH 5.5 and 1.02 mg Al/L for 24 h. Individuals were grouped according to their level of activity: ●, active (healthy) at $t = 0$ h; ○, active at $t = 24$ h; □, inactive (dying) at $t = 24$ h; ■, dead at $t = 24$ h; $^{**}P \leq 0.01$. For Cl/Na graph the dead daphnids were excluded in the calculations of the regression equation. For the Na/Al and Ca/Al graphs the live daphnids at $t = 0$ h were excluded from the regression equation.

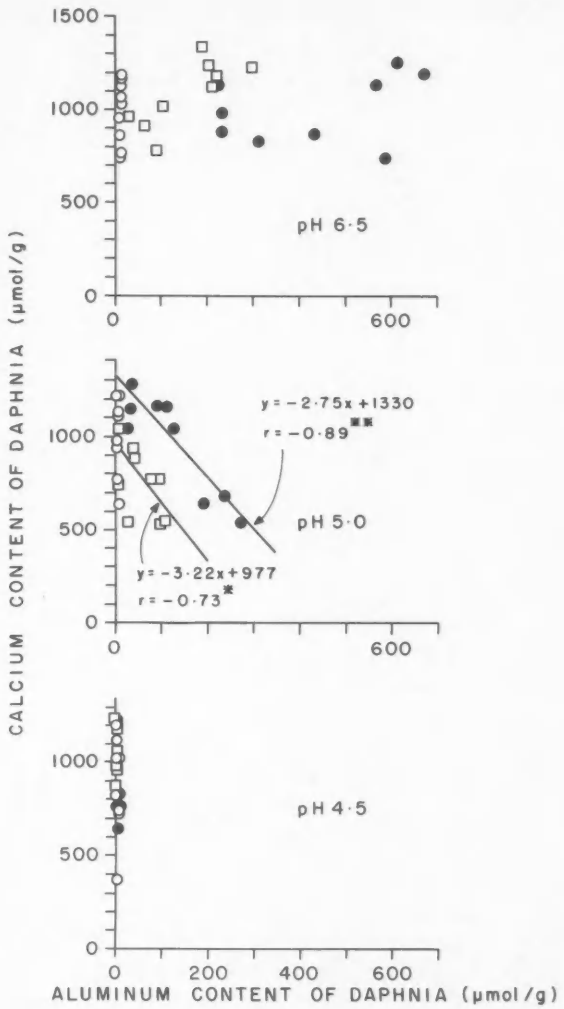

FIG. 5. Ca and Al content of *D. magna* as a function of pH and Al concentrations in the water. Each point represents the mean of 10 adult daphnids: ○, 0.02 mg Al/L; □, 0.32 mg Al/L; ●, 1.02 mg Al/L; $*P \leq 0.05$; $**P \leq 0.01$. Exposure times are the same as in Fig. 2.

µmol/g (4600 µg/g) at pH 5.0, and 150 µmol/g (3400 µg/g) at pH 4.5 (Fig. 2).

A large portion of the Na and Cl was lost prior to death. Individuals that were dying (i.e. inactive but still alive) lost more than 60% of their original Na and Cl content within 24 h (Fig. 3). There was a significant ($P \leq 0.01$) positive linear correlation between Na and Cl content, and between Na and Al content, and a negative correlation between Ca and Al content of *D. magna* (Fig. 4).

The Ca content of daphnids was highly variable and did not respond to either low pH or high Al concentrations in the water. Only a few individuals had slightly lower concentrations of Ca at low pH (≤5.5).

At both pH 5.5 (Fig. 4) and pH 5.0 (Fig. 5) there was a significant negative correlation between Al content and Ca content of daphnids exposed to 1.02 mg Al/L ($P \leq 0.01$) and to 0.32 mg Al/L ($P \leq 0.05$). This relationship did not exist at any

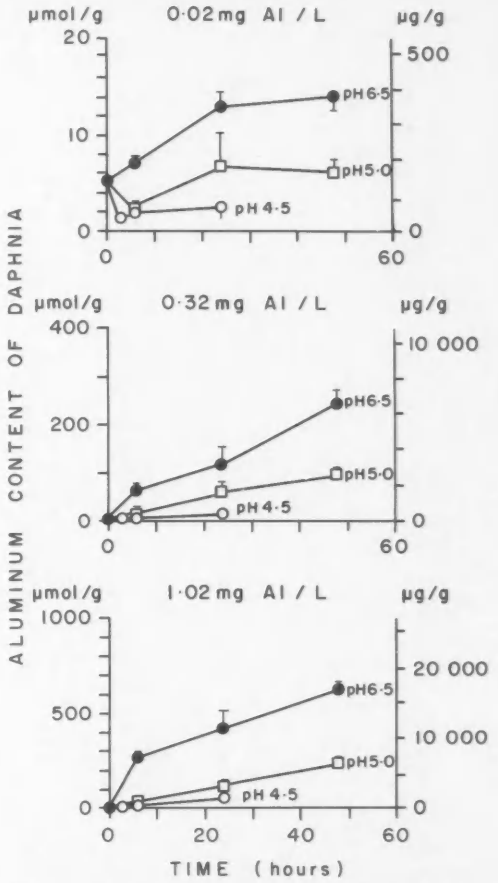

FIG. 6. Uptake of Al by *D. magna* at low pH and at elevated concentrations of Al in the water. Vertical bars represent 1 SD of three replicate samples each containing 10 adult daphnids.

of the other pHs, nor did it exist when the Ca concentration in the water was increased from 2.5 to 12.5 mg/L (data not shown).

Aluminum was taken up rapidly from the water by *D. magna*, particularly at pH 6.5 (Fig. 6). Aluminum concentrations as high as 415 µmol/g (11 200 µg/g) were recorded within 24 h at 1.02 mg Al/L, and these increased to 623 µmol/g (16 800 µg/g) within 48 h. Most of the Al at pH 5.0 and 6.5 was present as an insoluble aluminum hydroxide, $Al(OH)_3(s)$ (Table 1). This may have been ingested by the daphnids, or attached to the fine hairs on their thoracic legs, since concentrations of Al were slightly higher in active individuals compared with inactive (dying) or dead individuals (Fig. 3). The 24-h concentration ratio for Al (Al concentration in daphnids divided by the Al concentration in the water) was approximately 10 000, 4000, and less than 2000 at pH 6.5, 5.0, and 4.5, respectively (Table 2). Increasing the concentration of Ca in the water had no significant effect on the amount of Al taken up by the daphnids.

Discussion

Daphnia magna is extremely sensitive to low pH and died rapidly in soft water at and below pH 5.0 (Fig. 1). It is slightly

TABLE 1. Dominant forms of Al in the different treatments expressed as a percentage of the total Al.[a] Organoaluminum complexes are not included. Higher SO_4 concentrations are used at the lower pHs, since sulphuric acid was added to these treatments. An average fluoride concentration of 0.05 mg/L for surface water in this region was used. AlF_3, AlF_4, AlF_5, AlF_6, and $Al(SO_4)_2$ concentrations were insignificant (<0.1% of total Al). Values below 0.1% are indicated by dashes. Solid $Al(OH)_3$ was obtained by difference.

Experimental treatment		Al species (%)								
Total Al (mg/L)	pH	Al^{3+}	$AlOH^{2+}$	$Al(OH)_2^+$	$Al(OH)_4^-$	AlF^{2+}	AlF_2^+	$AlSO_4^+$	Total ionic Al	$Al(OH)_3$ solid
0.02	6.5	—	0.2	3.1	21.6	0.1	0.2	—	25.2	74.8
	5.0	3.1	3.2	2.0	—	48.6	41.8	0.4	99.1	0.9
	4.5	3.5	1.1	0.2	—	51.3	41.8	0.5	98.4	1.6
0.32	6.5	—	—	0.2	1.4	—	—	—	1.6	98.4
	5.0	9.5	9.3	6.2	—	16.9	1.7	1.2	44.8	55.2
	4.5	51.4	16.0	3.3	—	21.1	0.5	7.2	99.5	0.5
1.02	6.5	—	—	0.1	0.4	—	—	—	0.5	99.5
	5.0	3.0	2.9	1.9	—	5.3	0.5	0.4	14.0	86.0
	4.5	60.8	19.3	4.0	—	6.9	—	8.2	99.2	0.8

[a]These values were kindly provided by C. Driscoll (ALCHEM1) and are based on the chemical composition of Mirror Lake water and on the following constants:

	Reaction	log K
(1)	$Al^{3+} + H_2O = AlOH^{2+} + H^+$	−4.98
(2)	$Al^{3+} + 2H_2O = Al(OH)_2^+ + 2H^+$	−16.43
(3)	$Al^{3+} + 4H_2O = Al(OH)_4^- + 4H^+$	22.16
(4)	$Al^{3+} + F^- = AlF^{2+}$	7.02
(5)	$Al^{3+} + 2F^- = AlF_2^+$	12.76
(6)	$Al^{3+} + 3F^- = AlF_3$	17.03
(7)	$Al^{3+} + 4F^- = AlF_4^-$	19.73
(8)	$Al^{3+} + 5F^- = AlF_5^{2-}$	20.92
(9)	$Al^{3+} + 6F^- = AlF_6^{3-}$	20.87
(10)	$Al^{3+} + SO_4^{2-} = AlSO_4^+$	3.21
(11)	$Al^{3+} + 2SO_4^{2-} = Al(SO_4)_2^-$	5.11

more sensitive than *Daphnia catawba* (Havas and Likens 1985a) and considerably more sensitive than the crustaceans *Acantholeberis curvirostris* (Potts and Fryer 1979) and *Holopedium gibberum* (Havas and Likens 1985), which have been found in acidic environments (Sprules 1975; Potts and Fryer 1979; Confer et al. 1983).

The response of *D. magna* to Al seems to be more complex than its response to H^+. Aluminum may be either harmful or beneficial depending on its concentration and on the pH of the external solution. Maximum toxicity, attributable to Al, was observed at pH 6.5 (≥0.32 mg total Al/L) (Fig. 1). At this pH, 98% of the Al was theoretically present as an insoluble $Al(OH)_3(s)$ (Table 1). Other studies have also shown that maximum Al toxicity occurs when the water is saturated or oversaturated with Al (Baker and Schofield 1982; Havas and Likens 1985a). This can occur in natural systems following periods of high photosynthetic activity or liming, and can have a devastating effect on resident fish populations (Grahn 1980; Bengtsson et al. 1980; Dickson 1983) and presumably on other aquatic animals.

At pH 4.5, high concentrations of Al (1.02 mg/L) ameliorated H^+ toxicity although the effect was temporary (Fig. 1). Similar Al/H^+ amelioration was reported for survival of cleavage embryo of rainbow trout (*Salmo gairdneri*) at pH 4.5 (Holtze 1983), for survival of brown trout (*Salmo trutta*) at pH 3.5 and 4.0 (Brown 1981), and for survival of eggs of white suckers (*Catostomus commersoni*) and brook trout (*Salvelinus fontinalis*) between pH 4.2 and 4.8 (Baker and Schofield 1982).

At pH 4.5, approximately 99% of the Al was in solution. Pagenkopf (1983) suggested that trace metals compete with H^+ for the same exchange sites on the gill surface, which may account for some of the reduced toxicity of H^+. Also, polyvalent cations, such as $AlOH^{2+}$ and AlF^{2+} and possibly Al^{3+}, may be able to mimic Ca^{2+}, reduce membrane permeability, and thus protect the animals at low pH. More work is needed on the mechanisms responsible for Al/H^+ antagonism at low pH.

It is difficult to set an incipient lethal concentration for Al, since the chemical form of Al and thus its toxicity is strongly influenced by pH and by both organic and inorganic ligands (Baker 1982). The Ca content of the water can also influence the relative sensitivity of the biota and must be taken into consideration (Brown and Lynam 1982). With this in mind, the incipient lethal Al concentration for *D. magna* should be slightly less than 0.3 mg total Al/L in circumneutral, softwater lakes. This value is similar to the concentrations of Al (0.1–0.3 mg/L) that have been reported to be toxic to Atlantic salmon (*Salmo salar*), brown trout, white sucker, brook trout, and rainbow trout (Muniz and Leivestad 1980; Baker and Schofield 1982; Brown and Lynam 1982; Holtze 1983; van Coillie et al. 1983), but is much lower than the concentrations reported for the crustaceans *D. catawba* and *H. gibberum* (Havas and Likens 1985).

Effect of H^+ and Al on Salt Regulation

Both H^+ and Al interfered with salt regulation in *D. magna*. There was a net loss of total body Na and Cl both at low pH

TABLE 2. Concentration ratio (Al concentration in the biota divided by the Al concentration in the water at $t = 0$ h) for Al in *D. magna*. Daphnids were exposed to various concentrations of Al at different pHs for 24 h in water containing low (2.5 mg Ca/L) and high (12.5 mg Ca/L) concentrations of Ca. Values represent the mean ± SD of three replicate samples each consisting of 10 individuals. nd = no data.

Al (mg/L)	pH 6.5		pH 5.0		pH 4.5	
	Low Ca	High Ca	Low Ca	High Ca	Low Ca	High Ca
0.02	18 000±3500	18 000±6000	9000±8500	8500±4800	4300±1600	<2700
0.32	9 600±5800	nd	5200±2800	nd	1500±300	nd
1.02	11 000±4500	14 700±500	3000±400	3300±1700	1600±300	1200±400

(≤5.0) and at elevated concentrations of Al (≥0.32 mg/L) (Fig. 2). Dead and dying (inactive) individuals had significantly lower concentrations of Na and Cl than those which were active and otherwise healthy (Fig. 3). Daphnids had difficulty coordinating their appendages for both swimming and filter-feeding by the time total body Na concentrations dropped from 400 to 250 μmol/g at pH 6.5, to 200 μmol/g at pH 5.0, and to 150 μmol/g at pH 4.5. In a natural environment they would have difficulty surviving at this stage, since they would be unable to feed, to avoid predators, or to stay afloat. Once *Daphnia* stop swimming they sink rapidly.

Something in addition to the loss of Na (and Cl) may be responsible for the decreased survival of the daphnids at pH 6.5 compared with pH 4.5, since comparable body Na concentrations corresponded to different levels of activity at these two pHs. For example, with an average Na concentration of 150 μmol/g, 80–100% of the individuals remained active at pH 4.5 compared with 40–60% at pH 6.5. In brown trout, respiratory stress attributed to the buildup of mucus on the gills is an additional stress at higher pHs (Muniz and Leivestad 1980). Whether this also occurs in *D. magna* or in other invertebrates is not known.

At pH 4.5, Al temporarily prolonged survival of *D. magna* (Fig. 1) and reduced the net loss of both Na and Cl (Fig. 2). Havas and Likens (1985b) found that for *D. magna*, Al reduced ^{22}Na outflux at low pH but had no significant effect on ^{22}Na influx, which remained low. This would account for the beneficial but temporary effect of Al at low pH.

Studies with brown trout (Muniz and Leivestad 1980) and rainbow trout (Holtze 1983) indicate that both Al and H^+ interfere with salt regulation. The net loss of plasma Cl from brown trout was observed at and below pH 5.1 and as low as 0.19 mg Al/L. At lower pHs (pH 4.0), just as in the present study, Al reduced the net loss of Cl (Muniz and Leivestad 1980). It seems, therefore, that *D. magna*, brown trout, and rainbow trout have a similar physiological response to both low pH and elevated concentrations of Al.

Increasing the Ca content of the water from 2.5 to 12.5 mg/L also prolonged survival in some of the treatments (Fig. 1) and reduced the net loss of Na and Cl from the daphnids. McWilliams and Potts (1978) found that Ca changed the electrical potential across the gill membrane of brown trout, which reduced the permeability of the gill to the passive fluxes of H^+ and Na^+. As a consequence, the passive diffusion of Na from the gills to the external medium decreased and the fish were able to survive longer at higher Ca concentrations. Calcium reduced membrane permeability at and above pH 5.0 but had no significant effect at lower pHs, just as in this study.

McDonald (1983b) observed a similar response in rainbow trout. The net loss of plasma Na and Cl at low pH was greater at low Ca concentrations (223 μeq/L, 4 mg/L) than at higher Ca concentrations (1930 μeq/L, 35 mg/L). It is now well documented that Ca affects both diffusional losses and active uptake of Na (McDonald 1983a; Havas et al. 1984) and can protect organisms at low pH and in the presence of trace metals (Pagenkopf 1983).

In contrast with the changes in Na and Cl content of the daphnids, neither Al nor low pH significantly altered the Ca content of the daphnids. However, since there was no attempt to standardize molting, subtle changes in Ca content may have been missed. The only exception to this was observed at pH 5.5 (Fig. 4) and at pH 5.0 (Fig. 5) with 0.32 and 1.02 mg Al/L. At these pHs there was a significant ($P \leq 0.05$) negative correlation between Ca and Al content of the *Daphnia*. Increasing the Ca concentration in the water destroyed this relationship. Why this negative correlation is limited to such a narrow range in pH is not known. I found that Ca was not lost at higher pHs and Al bioaccumulation was reduced at lower pHs, which suggests that this negative relationship between Al and Ca content in *D. magna* may be coincidental rather than causal.

Calcium metabolism may be influenced as much, if not more, by low pH as by elevated concentrations of Al. Malley et al. (1985) found that active uptake of ^{45}Ca by the crayfish (*Orconectes virilis*) was inhibited at pH 5.5 in the presence of Al. However, only 20% of the ^{45}Ca inhibition was attributed to Al, while 80% was attributed to H^+.

Although Ca is more tightly bound than either Na or Cl and is lost less rapidly, subtle changes in Ca regulation may have a significant effect on the organism. Nelson (1982) examined the effect of pH and Ca on rainbow trout and found that while body Ca levels in the trout did not decrease at low pH, there was a reduction in ossification of teeth and branchiostegals. Similarly, McWilliams (1983) found that surface-bound Ca was removed more rapidly from the gills of brown trout in acidic than in neutral solutions. Such subtle changes may have a significant effect on the organism but may be masked if total body Ca concentration are compared, as in the present study.

Bioaccumulation of Al by *D. magna* is pH dependent with maximum uptake at pH 6.5 and minimum uptake at pH 4.5 (Table 2). Concentrations of Al in the daphnids increased from 350 μg/g in the reference treatment (pH 6.5, 0.02 mg Al/L) to 3000 and 11 000 μg/g at 0.32 and 1.02 mg Al/L, respectively. At pH 5.0 and 4.5, the same pattern was observed (i.e. increasing Al content in daphnids with increasing Al concentration in the water) although the actual concentrations of Al in the *Daphnia* were considerably lower.

Crayfish, cisco (*Coregonus albua*), pumkinseed (*Lepomis gibbosus*), and rainbow trout also accumulate Al from the water

(Grahn 1980; Harvey et al. 1982; Malley et al. 1985). In fish, most of this Al seems to be associated with gills rather than internal organs.

In *D. magna*, Al appears to be attached to the fine hairs on the thoracic legs, adsorbed onto the carapace and onto the chloride cells, and taken up into the hind gut through the anal opening (M. Havas and T. C. Hutchinson, unpubl. data). In fairy shrimp and diptera larvae, Al appears to be associated with chloride cells (M. Havas and T. C. Hutchinson, unpubl. data), which are believed to be the sites of active uptake of Na and Cl from the water (Komnick 1977).

In conclusion, Al may be either harmful or beneficial to *D. magna* in soft water. Aluminum interferes with Na, Cl, and possibly with Ca regulation. It is taken up rapidly, particularly at circumneutral pHs, and may be an additional source of Al for zooplanktivorous fish and other predatory animals. Aluminum has been shown to influence the distribution of fish in acid-sensitive lakes, but to what extent it has influenced the distribution of aquatic invertebrates in these lakes is not known.

Acknowledgments

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Inter- and Intra-specific Variability in the Response of Zooplankton to Acid Stress[1]

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Price, E. E., and M. C. Swift. 1985. Inter- and intra-specific variability in the response of zooplankton to acid stress. Can. J. Fish. Aquat. Sci. 42: 1749–1754.

We measured the toxicity of sulfuric acid to natural populations of *Daphnia pulex*, *D. galeata mendotae*, *Simocephalus serrulatus*, *Mesocyclops edax*, *Chaoborus americanus*, and *C. punctipennis*. Organisms were collected in the spring and fall from acidic (pH 5.4) and circumneutral (pH 6.3–7.5) ponds, and their response to low pH was compared in 48- or 96-h acute bioassays. Based on 48- and 96-h LC50, cladocerans were most susceptible to acid stress, followed by *Mesocyclops* and *Chaoborus* larvae. *Simocephalus* was the most tolerant cladoceran, followed by *D. pulex* then *D. galeata mendotae*. *Daphnia* populations tested in the spring or early summer were more tolerant of low pH than those tested in the fall. *Mesocyclops edax* from an acid pond were more tolerant than those from a neutral pond. The response of the two *Chaoborus* species to low pH were quite similar; their 96-h LC50 (2.00, 2.09) was two pH units lower than those of cladocerans and one pH unit lower than that of *M. edax*. Our data demonstrate interspecific variability in acid sensitivity and suggest intraspecific variability due to habitat and season.

Les auteurs ont quantifié la toxicité de l'acide sulfurique pour les populations naturelles de *Daphnia pulex*, *D. galeata mendotae*, *Simocephalus serrulatus*, *Mesocyclops edax*, *Chaoborus americanus* et *C. punctipennis*. Au printemps et à l'automne, ils ont recueilli des organismes dans des étangs acides (pH 5,4) et presque neutres (pH 6,3–7,5); leur réaction à un faible pH a été comparée au cours de bio-essais aigus de 48 h et de 96 h. D'après la CL50 après 48 h et 96 h d'exposition, les cladocères étaient plus sensibles au stress acide; venaient ensuite les larves de *Mesocyclops* et de *Chaoborus*. Les plus tolérants étaient *Simocephalus* puis *D. pulex* et *D. galeata mendotae*. Les populations de *Daphnia* étudiées au printemps ou au début de l'été étaient plus résistantes à un faible pH que celles étudiées à l'automne. Quant à *M. edax*, les individus provenant d'un étang acide étaient plus tolérants que ceux peuplant un étang neutre. La réaction des deux espèces de *Chaoborus* à un faible pH était très semblable : leur CL50 après 96 h d'exposition (2,00, 2,09) était deux unités de pH moins élevée que celle des cladocères et une unité de pH moins élevée que celle de *M. edax*. Les données recueillies révèlent une variabilité interspécifique de la sensibilité à l'acide et portent à croire à une variabilité intraspécifique entraînée par l'habitat et la saison.

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Acidification of freshwater caused by acid precipitation is a problem in Europe and North America (Almer et al. 1974; Glass et al. 1979) which should continue to increase as the use of fossil fuels increases (Likens et al. 1979). The acidification phenomenon makes the effect of pH on zooplankton communities of considerable interest because of the importance of zooplankton in the structure and function of lacustrine communities.

Surveys have shown that acidification simplifies zooplankton communities. Sprules (1975) found a sharp decrease in the abundance and diversity of zooplankton below pH 5.0. Hendrey and Wright (1976) and Confer et al. (1983) found fewer zooplankton species at lower pH in Norwegian lakes and in lakes in northeastern North America. Bible (1971), Almer et al. (1974), and Janicki and DeCosta (1979) all found significantly lower zooplankton density and diversity in acidified lakes. These survey studies do not provide much information on the response of individual species to acid stress.

Some information on the variability in response of individual species to acid stress is available. Havas and Hutchinson (1982) used bioassays to determine mortality curves of crustaceans (*Daphnia*, *Lepidurus*, *Branchinecta*) and insect larvae (*Orthocladius*, *Limnephilus*, *Chironomus*). The crustaceans, all from alkaline ponds, died below pH 4.5 but their mortality curves varied. However, *Orthocladius* and *Limnephilus* from the same alkaline ponds survived extended periods at pH 3.5, and *Chironomus* from acidic ponds survived at pH 2.8 and 8.2. Bible (1971) showed that the copepod *Mesocyclops edax* could survive a wide range of environmental pH in Cheat Lake, West Virginia. Most measurements of *Daphnia*'s tolerance to acid have been obtained using laboratory-reared populations. Davis and Ozburn (1969) determined a survival range for *D. pulex* between pH 4.2 and 10.4. Parent and Cheetham (1980) found 85% survival of *D. magna* above pH 4.5 and less than 50% survival below pH 4.2. Walton et al. (1982) found 80–100% survival of *D. pulex* above pH 4.3.

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TABLE 1. Site characteristics of sources of bioassay organisms (pH and temperature measurements and organism collection were done on the tabulated date).

Site[a]	Date collected	Species	pH	Temperature (°C)	Ca^{2+} ($mg \cdot L^{-1}$)
Pond A	16 Sept. 1982 (fall)	*D. pulex*	6.3	18.0	2.2[b]
	28 Apr. 1983 (spring)	*D. pulex*	7.0	16.0	
Pond B	2 Sept. 1982 (fall)	*S. serrulatus*	7.0	17.0	7.2[b]
	14 Apr. 1983 (neutral)	*C. americanus*	6.3	18.0	
Pond C	5 June 1983 (neutral)	*M. edax*	6.7	18.0	2.4[b]
Lake Habeeb	26 Aug. 1983 (fall)	*D. g. mendotae*	7.0	22.0	7.8[b]
	17 June 1983 (spring)	*D. g. mendotae*	7.5	18.0	
Crane Pond	8 Jan. 1983 (acid)	*M. edax*	5.4	15.0	1.6[c]
	8 Jan. 1983 (acid)	*C. punctipennis*	5.4	15.0	

[a]Pond A: Frostburg Road, Garrett Co., MD (39°39′38″N, 78°59′29″W); Pond B: Old Frostburg Road, Garrett Co., MD (39°39′50″N, 79°00′W); Pond C: Beall School Road, Garrett Co., MD (39°40′47″N, 78°58′41″W); Lake Habeeb: Rocky Gap State Park, Allegany Co., MD (39°42′13″N, 78°39′30″W); Crane Pond: Masontown Road, Newport, NC (34°48′16″N, 76°59′20″W).
[b]Ca^{2+} sample collected on 10 Sept. 1983.
[c]Ca^{2+} sample collected on 10 Jan. 1984.

Our study was designed to answer the following questions about zooplankton response to acid stress: (1) Does tolerance to acid stress vary among taxa? (2) Does tolerance to acid stress vary with the pH of the pond from which the population is collected — are populations from acid ponds more tolerant than populations from circumneutral ponds? (3) Are there seasonal differences in tolerance of acid stress within taxa? To answer these questions we measured acid tolerance of several zooplankton taxa collected from both acid and neutral ponds and in spring and fall.

Methods

Water and Animal Collection and Preparation

Bioassays were performed in 38-L aquaria containing 20 L of pond water that had been filtered through a 5-μm filter bag. Experimental pH's were obtained following Neville's (1979) procedure: acidification with reagent-grade H_2SO_4 to the lowest experimental pH, aeration for 24 h, and titration to the experimental pH with reagent-grade NaOH. Unacidified pond water was used as a control pH treatment in each bioassay. Calcium ion concentration in the ponds varied from 1.6 to 7.8 $mg \cdot L^{-1}$ (AA, Perkin Elmer model 306) (Table 1); these values lie in the hard water category with respect to the biotic affects of acidification (Graham and Wood 1981; Wood and McDonald 1982). A Beckman pH meter (model 1019) was used to determine pH during all experiments; experimental and control pH's varied <0.1 pH unit during the bioassays.

All bioassays were run in an incubator at ±0.5° of the collection temperature. Experimental temperatures ranged from 15 to 22°C (Table 1). For any within-species comparison the greatest temperature difference was 3°. The photoperiod was 16 h light : 8 h dark with light provided by one Vita-lite (Duro-test) 14-W bulb above each aquarium. The water in the experimental aquaria or beakers was not aerated during the bioassays nor were the test organisms fed. Environmental Protection Agency standards for measuring the acute toxicity of effluents to aquatic organisms were followed during all experiments (Peltier 1978).

Animals for the bioassays were collected from circumneutral habitats in western Maryland and an acid pond in North Carolina. *Daphnia pulex*, *Simocephalus serrulatus*, *Chaoborus americanus*, and *Mesocyclops edax* were collected from three small farm ponds in Garrett County, Maryland. *Daphnia galeata mendotae* was collected from Lake Habeeb, located at Rocky Gap State Park, Allegany County, Maryland. The farm ponds are small (~0.25 ha) and shallow (~2 m); Lake Habeeb is larger (~20 ha) and deeper (20 m). These impoundments support diverse zooplankton communities and large fish populations. *Chaoborus punctipennis* larvae and *M. edax* were collected in Crane Pond, Newport, North Carolina. Crane Pond is an acidic dystrophic pond (1 ha, 3.5 m). It contains diverse zooplankton and fish faunas (M. C. Swift, unpubl. data). The collection sites varied in pH, Ca^{2+} concentration, and time of collection (Table 1). Zooplankton were collected with horizontal hauls using a 240-μm zooplankton net. Plankton were sieved through a 500-μm sieve after each haul to separate the largest organisms, which were then transported to the laboratory and sorted by species into filtered water from the collection site using the keys of Edmondson (1959). All bioassay organisms were acclimated to laboratory conditions for 24 h in an incubator maintained within ±0.5° of the collection site temperature and on a 16 h light : 8 h dark photoperiod. They were not fed.

Bioassays and Analysis

A 48- or 96-h acute bioassay was used to determine the sensitivity of zooplankton to reduce pH. The pH range for each group of organisms was determined by a preliminary pH screening test and was selected to include pH values causing 100% mortality and no mortality. All cladocerans were tested at pH 4.0, 4.2, 4.4, 4.7, 4.9, 5.2, 5.4, 5.7, and 6.0. The two populations of *M. edax* were tested at pH 2.7, 3.1, 3.3, 3.5, 3.7, 3.9, 4.2, 4.4, and 5.0. *Chaoborus* were tested at a pH range of 1.6–2.6 in increments of 0.2 pH units. Cladocerans and copepods were held individually in modified tissue culture trays (Costar 3524) containing 24 wells per tray during experiments. Trays were modified by removing the plastic cover on each tray and replacing it with fine mesh which allowed circulation between each well and the treatment tank. Each treatment

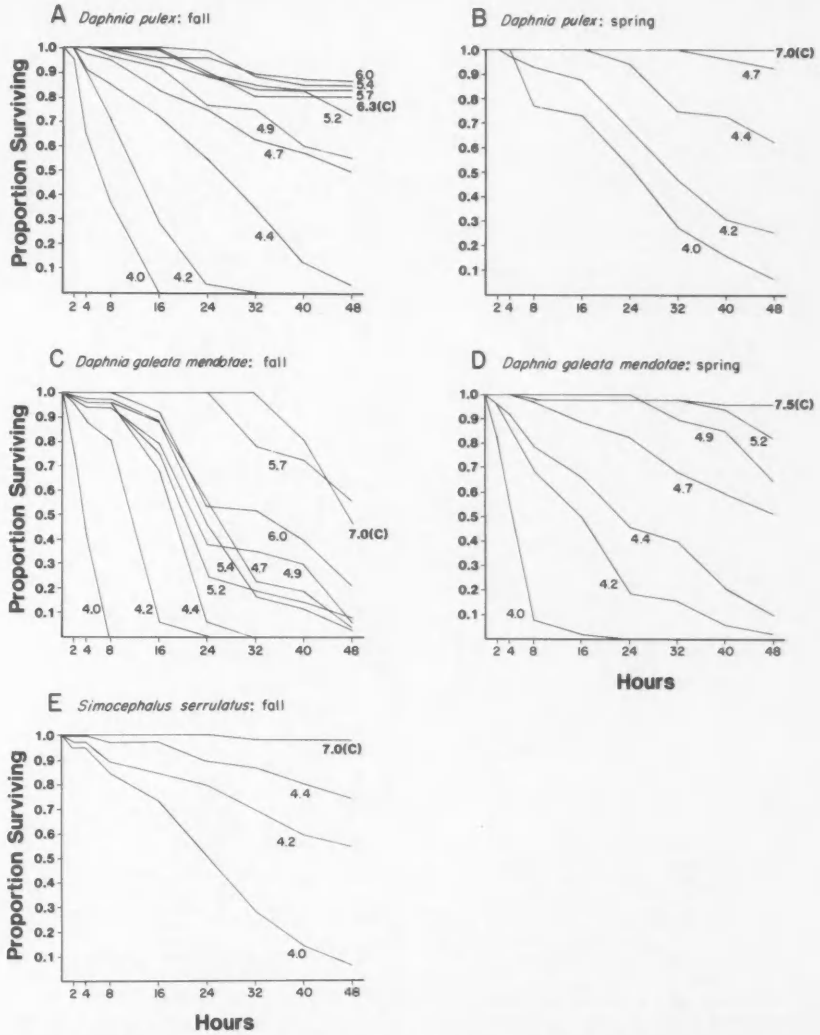

FIG. 1. Survival of cladocerans exposed to pH 4.0–6.0 in a 48-h acute bioassay. Data points are connected by lines for convenience. The numbers beside each line are the test pH values; the unacidified control response is labeled C. (A) Fall *D. pulex*; (B) spring *D. pulex*; (C) fall *D. galeata mendotae*; (D) spring *D. galeata mendotae*; (E) fall *S. serrulatus*.

contained two trays (48 individuals). Bioassays on *Chaoborus* were conducted using 200-mL beakers containing five larvae each with four replicates in each treatment (20 individuals). During a bioassay, cladocerans and copepods were observed for death at 2, 4, 8, 16, 24, 32, 40, and 48 h. This pattern was followed for *Chaoborus* with additional observations after 48 h at 8-h intervals until 96 h. Tissue culture trays were checked by observing each individual well with a dissecting microscope. Lack of movement was used as the criterion of death (Anderson 1944).

Percent mortality was plotted against observation time to show the time course of mortality in each bioassay. We used probit analysis (Finney 1977) and the trimmed Spearman–Karber method (Hamilton et al. 1977) to determine LC50 values (cladocerans and copepods, 48 h; *Chaoborus*, 96 h) and 95% confidence intervals. The 95% confidence intervals of the LC50 values were used to test for differences in LC50 among all taxa; confidence intervals that overlapped were considered not significantly different.

Results

The fall and spring populations of *D. pulex* responded differently to reduced pH. There was no survival at pH 4.0 and 4.2 and greater than 80% survival above pH 5.2 within 48 h for the fall population of *D. pulex* (Fig. 1A). In contrast, there was some survival of the spring population at both pH 4.0 and 4.2 and greater than 80% survival above pH 4.7 (Fig. 1B). The 48-h LC50 values for the fall and spring populations were pH 4.71 and 4.37, respectively, and were significantly different.

The two populations of *D. galeata mendotae* were very different in their response to reduced pH. In the fall population,

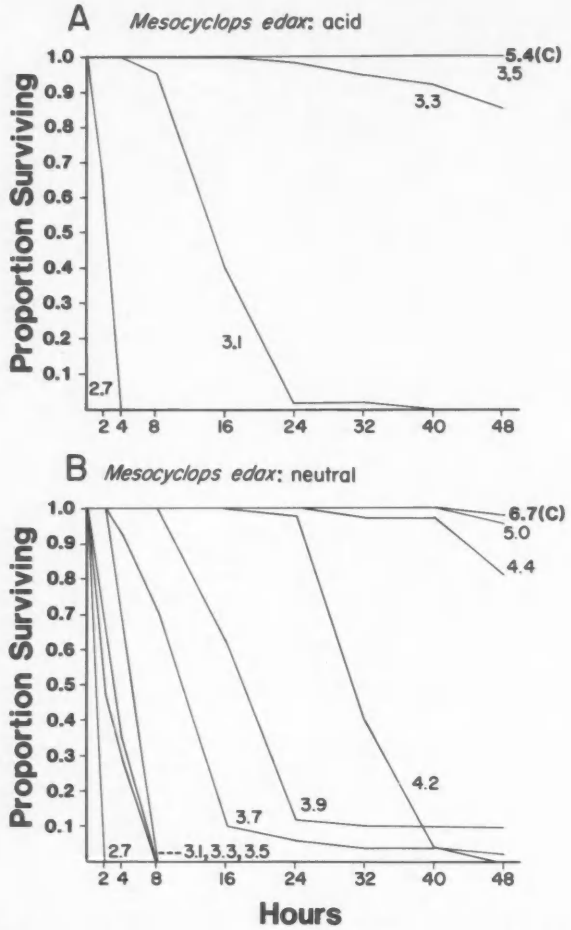

FIG. 2. Survival of *M. edax* from (A) acid and (B) neutral ponds exposed to pH 2.7–5.0 in a 48-h acute bioassay. Data points are connected by lines for convenience. The numbers beside each line are the test pH values; the unacidified control response is labeled C.

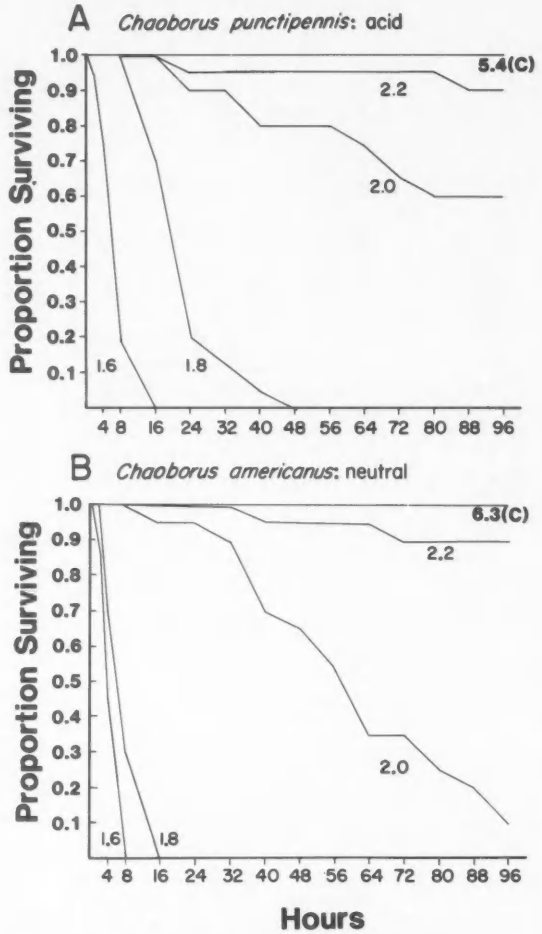

FIG. 3. Survival of (A) *C. punctipennis* and (B) *C. americanus* exposed to pH 1.6–2.6 in a 96-h bioassay. Data points are connected by lines for convenience. The numbers beside the lines are the test pH values; the unacidified control response is labeled C.

complete mortality occurred at and below pH 4.4. Between pH 4.7 and 5.4 the time course of mortality varied but the 48-h survivorship was very low (Fig. 1C). Above pH 5.4 there was considerable mortality including the unacidified control treatment at pH 7.0. The spring population exhibited little survivorship at pH 4.0 and 98 and 90% mortality at pH 4.2 and 4.4, respectively. There was greater than 80% survival above pH 5.0 (Fig. 1D). Due to the high mortality in the fall population, an LC50 value could not be calculated. The 48-h LC50 value for the spring population was 4.77.

Simocephalus serrulatus had the lowest 48-h LC50 (pH 4.19) of the cladocerans we tested. This population survived at pH 4.0 and 4.2 for 48 h (Fig. 1E). At pH 4.2, 45% of the *S. serrulatus* died compared with 75% of the spring *D. pulex*, the second most tolerent cladoceran.

The responses of *M. edax* from acidic Crane Pond and *M. edax* from circumneutral Pond C were quite different. *Mesocyclops edax* from Crane Pond was the more tolerant population; complete mortality occurred below pH 3.1, and greater than 80% survival occurred above pH 3.3 (Fig. 2A). *Mesocyclops edax* from Pond C exhibited complete mortality below pH 3.5, less than 10% survival between pH 3.7 and 4.2, and greater than 80% survival above pH 4.4 (Fig. 2B). The 48-h LC50 values for the Crane Pond and Pond C populations, pH 3.22 and 4.36, respectively, were significantly different.

The responses of *C. punctipennis* from acidic Crane Pond and *C. americanus* from Pond C were quite similar. Complete mortality was observed for *C. punctipennis* at pH 1.6 and 1.8 at 16 and 48 h, respectively (Fig. 3A). There was complete mortality of *C. americanus* at pH 1.6 and 1.8 at 8 and 16 h, respectively (Fig. 3B). At pH 2.0, more *C. punctipennis* survived (60%) than *C. americanus* (10%). The 96-h LC50 values for *C. punctipennis* and *C. americanus* were pH 2.00 and 2.09, respectively. Although the pattern of mortality varied between these two species, their LC50 values were not significantly different.

Based on the LC50 values, *Chaoborus*, larvae were the most resistant to acid stress, followed by *M. edax* from acidic Crane Pond, *Simocephalus*, *M. edax* from neutral Pond C and spring *D. pulex*, and fall *D. pulex* and spring *D. galeata mendotae*

TABLE 2. Acute toxicity of sulfuric acid to natural zooplankton populations. The pH values tabulated are LC50 values and their 95% confidence intervals from 48- and 96-h acute bioassays. The species tested are arranged in order of decreasing LC50. The 48-h LC50 for fall *D. galeata mendotae* could not be calculated but is above that of spring *D. galeata mendotae*.

Species	48-h LC50 (pH)	96-h LC50 (pH)	95% confidence interval (pH)
D. galeata mendotae (fall)			
D. galeata mendotae (spring)	4.77[a]		4.70–4.86
D. pulex (fall)	4.71[a]		4.64–4.83
D. pulex (spring)	4.37[a]		4.31–4.42
M. edax (neutral)	4.36[b]		4.31–4.42
S. serrulatus (fall)	4.19[a]		4.14–4.23
M. edax (acid)	3.22[a]		3.20–3.25
C. americanus (neutral)		2.09[a]	2.04–2.13
C. punctipennis (acid)		2.00[a]	1.94–2.06

[a]Calculated using probit analysis method.
[b]Calculated using trimmed Spearman–Karber method.

(Table 2). The 48-h LC50 of fall *D. galeata mendotae* could not be calculated because of high control mortality but it appears to be above that of spring *D. galeata mendotae*.

Discussion

Descriptive studies of zooplankton community structure in acidified lakes have documented decreases in number, diversity, and biomass (e.g. Sprules 1975, 1977; Yan and Strus 1980; Confer et al. 1983; Brezonik et al. 1984; Keller and Pitblado 1984). Based on his work with fish communities, Beamish (1974) suggested that the response of individual species to acid stress is variable, indicating different thresholds of tolerance. Our results for zooplankton provide experimental evidence to support his suggestion that species vary in their response to acid stress. The variability in response to acid stress measured in our experiments helps explain the patterns of zooplankton distribution in acidified lakes.

Although most zooplankton communities in acid lakes include some cladocerans, as a group they are characterized as acid sensitive. Both *D. pulex* and *D. galeata mendotae* are apparently very sensitive to acid stress (48-h LC50 ≥ pH 4.37). Yan and Strus (1980) found neither species in temperate acidified lakes; Keller and Pitblado (1984) found them in a few acidified lakes but their frequency of occurrence and relative abundance were low. *Simocephalus* is not often abundant in zooplankton collections and its distribution with respect to pH is not well known. It was, however, the most acid-tolerant cladoceran we tested, and it was present in an acid pond in Florida (Brezonik et al. 1984).

The difference in 48-h LC50 between *M. edax* populations from acid and neutral ponds (pH 3.22 and 4.36, respectively) suggests a large degree of variability among populations in response to acid stress. These results help explain the wide distribution of *M. edax* with respect to pH in North America. *Mesocyclops edax* has been characterized as a common acid-tolerant species in Florida (Brezonik et al. 1984), as an indicator of lakes in the initial stages of acidification (Sprules 1975, 1977), and as more successful at pH 6–7 than at pH 3.6–4.0 (Bible 1971). The extent to which the ability to survive a wide range of environmental pH reflects genetic differences or simply physiological adaptation is unknown. However, Wyngaard (1982) has demonstrated heritable genetic differences in life history parameters between *M. edax* populations from Florida and Michigan.

Insect larvae appear to be much more resistant to acid stress than either copepods or cladocerans. Our data agree with this pattern, as do the studies of Havas and Hutchinson (1982) on *Orthocladius*, *Limnephilus*, and *Chironomus* and Stockinger and Hayes (1960), Eriksson et al. (1980), and Yan et al. (1982) on *Chaoborus*. Differences in the pattern of mortality between acid- and neutral-adapted populations of *Chaoborus* larvae were also apparent. The between-habitat comparison using *Chaoborus* was less robust than the *Mesocyclops* experiments because two different species were used. However, the population from acidic Crane Pond survived longer at each test pH below pH 2.0. These results suggest that interspecific differences in resistance to acid stress exist in *Chaoborus* also.

The effect of temperature on the response of zooplankton to acid stress is unknown. Data from fish studies are ambiguous (Spry et al. 1981); in some cases, survival was greater at low temperature (~4°C) than at high temperature (~20°C), but acclimation to higher temperatures modified this pattern. In our study the difference in temperature between bioassays of the same species (*D. pulex*, *D. galeata mendotae*, *M. edax*) or genus (*Chaoborus*) were no greater than 3°C. We feel that the effects of differences in habitat pH (acid, neutral) or season (spring, fall) are more likely to affect response to acid stress than the small differences in experimental temperature.

The mechanism responsible for the seasonal variability in response to acid stress we measured in *D. pulex* and *D. galeata mendotae* is not clear. The fall population of *D. pulex* was significantly more sensitive to acid stress than the spring population (48-h LC50 pH 4.71 and 4.37, respectively). This seasonal difference was even more pronounced in *D. galeata mendotae* in which there was so much mortality in the fall population (54% even in the unacidified control) that the 48-h LC50 could not be calculated. This increased sensitivity may have been caused by lack of food. Food availability affects the sensitivity of *Daphnia* to toxicants (Biesinger and Christensen 1972; Winner and Farrell 1976). If phytoplankton was less abundant in the fall, one would expect fall *D. pulex* and *D. galeata mendotae* populations to be food stressed and therefore less resistant to acid stress than the spring populations. This hypothesis will require further testing.

Most acute laboratory studies on the tolerance of zooplankton to reduced pH have reported their results as "critical pH" values. These values have been defined as the highest pH value where survival was less than 50% (Parent and Cheetham 1980), as the lowest pH value where survival was greater than 80% (Walton et al. 1982), or as the highest pH value below which the rate of mortality increased significantly compared with the control (Havas and Hutchinson 1982). To facilitate comparison with their results, we calculated critical pH, following Walton et al. (1982), as the lowest pH value where survival was greater than 80%. Those studies that used laboratory-cultured juvenile or adult *D. pulex* or *D. magna* (Davis and Ozburn 1969; Parent and Cheetham 1980; Walton et al 1982) reported critical pH values around 4.2 for *D. pulex* and *D. magna*. Our acute bioassays, using natural populations, resulted in a range of critical pH values for cladocerans from pH 4.7 to 5.4 (fall *S. serrulatus*, 4.7; spring *D. pulex*, 4.7; spring *D. galeata mendotae*, 5.2; fall *D. pulex*, 5.4). Havas and Hutchinson (1982) measured a critical pH of 4.5 for natural populations of *D. middendorffiana*. The discrepancy between results from cultured and natural popu-

lations suggests that the actual "critical pH" for cladocerans is higher than previously reported.

Standardized *Daphnia* bioassay procedures require that the test organisms be fed ad libitum and maintained in optimum reproductive condition before each experiment (Goulden et al. 1982). This procedure is designed to guarantee that the only effect observed in the bioassay is due to the toxicant. Conflicting results of acute acid bioassays using natural and cultured populations of cladocerans argue for the use of both these population types in toxicity studies. Bioassays using cultured organisms in standardized, defined physiological condition are appropriate for determining the relative toxicity among toxicants. However, this type of bioassay may provide little information about in situ effects of a toxicant on natural populations. Natural populations should be used to examine the ecological effects of toxicants. Caution is necessary when using natural populations; food availability and adaptation to local conditions might affect tolerance limits. Indeed, Buikema et al. (1980) commented that any attempt to relate effects observed in the laboratory to field situations may be meaningless because in natural environments, algae are not available ad libitum.

We measured the tolerance of some zooplankton populations to reduced pH in this study. Additional acute studies on other species are needed to discover the range of variability in zooplankton's response to acidification. At best, however, acute bioassays only provide an indication of the tolerance of a particular life history stage. In order to determine the effects of acidification on the lake ecosystem, chronic studies of the trophic structure of lakes under acid stress are required.

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Effect of Female Size, Egg Size, and Water Temperature on Developmental Biology of Chum Salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia

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Beacham, T. D., and C. B. Murray. 1985. Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia. Can. J. Fish. Aquat. Sci. 42: 1755–1765.

We incubated eggs and alevins for five chum salmon (*Oncorhynchus keta*) families in each of three female size classes under controlled water temperatures of 4, 8, and 12°C. Egg survival from fertilization to hatching was highest for eggs from small females and lowest for eggs from large females at all three incubation temperatures. Egg survival was highest at 8°C, and at 4°C survival rates were largely determined from fertilization to epiboly, but at 12°C the lowest egg survival rates were recorded from eye pigmentation to hatching. There were marked differences in egg survival rates among families. Overall survival rates from egg fertilization to fry emergence were most divergent between the large and small female size class at 12°C. Hatching time of the alevins and emergence time of the fry were similar among the three female size classes at each incubation temperature. Alevins hatching at 4°C were the longest, but those hatching at 12°C were the heaviest. Larger females produced alevins with both more yolk reserves and more body tissue at hatching than those from smaller females. The relation between egg weight and subsequent alevin size was dependent upon incubation temperature. At emergence, the longest and heaviest fry were produced by the largest female size class.

Des œufs et des alevins vésiculés provenant de cinq familles de saumon kéta (*Oncorhynchus keta*) réparties en trois classes de longueur de femelles ont été gardés en incubateur dont la température de l'eau était maintenue à 4, 8 et 12°C. À ces températures, la survie des œufs, de la fertilisation à l'éclosion, était plus élevée chez ceux provenant de petites femelles, et plus faible chez ceux issus de grosses femelles. La survie était plus élevée à 8°C tandis qu'à 4°C, les taux de survie étaient en grande partie fixés de la fertilisation à l'épibolie; à 12°C, les plus faibles taux de survie ont été observés de la pigmentation des yeux à l'éclosion. Il y avait des différences marquées entre les taux de survie des œufs selon la famille. Pour ce qui est des petites et des grosses femelles, la variabilité des taux de survie globaux de la fertilisation à l'émergence des alevins était plus élevée à 12°C. Le moment de la sortie des alevins vésiculés et de l'émergence des alevins nageants était semblable chez les trois classes de longueur de femelles à chaque température d'incubation. Les alevins vésiculés gardés à 4°C étaient les plus longs tandis que ceux qui étaient maintenus à 12°C étaient les plus gros. Par rapport aux petites femelles, les grosses femelles ont produit des alevins vésiculés possédant de plus importantes réserves vitellines et plus de tissu corporel à l'éclosion. La relation entre le poids des œufs et la taille ultérieure des alevins vésiculés dépendait de la température à l'incubation. À l'émergence, les plus gros et les plus longs alevins nageant provenaient de la classe des plus longues femelles.

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In British Columbia, chum salmon (*Oncorhynchus keta*) return to their natal rivers to spawn at 3–5 yr of age (Pritchard 1943; Ricker 1980; Beacham and Starr 1982). Body size variation in both sexes in a spawning population may be considerable, especially for males (Beacham and Murray 1985). Females within a spawning population may differ in postorbital–hypural length by 200 mm, and size variation among female salmon may be an important component of subsequent egg survival (Allen 1957; van den Berghe and Gross 1984). If egg mortality is related to female size, then variation in female size may be an important component of population dynamics of a stock.

Size of salmonid alevins and fry may be a significant determinant of subsequent growth and survival (Bams 1969; Fowler 1972). Size of alevins and fry may be influenced by the size of eggs from which they originate, with larger alevins and fry derived from larger eggs (Smirnov 1975; Kazakov 1981; Wallance and Aasjord 1984), but this may not always be the case (Privol'nev 1960; Privol'nev et al. 1964; Beacham et al. 1985). Alevins and fry derived from large eggs are bigger because of the greater amounts of yolk in large eggs than in small ones (Smirnov 1975). However, the size of alevins and fry from eggs of different size is dependent upon the efficiency of conversion of yolk to body tissue, and conversion efficiencies are dependent upon water temperatures during incubation (Marr 1966; Heming 1982). Whether or not larger eggs produce larger

alevins and fry may thus be dependent upon the temperature regime during incubation.

We investigated the effect of female body length on egg and alevin survival under three controlled temperature regimes for a Vancouver Island chum salmon stock. The effect of initial egg size on subsequent alevin and fry size under each temperature regime was also investigated to determine if previous conflicting results were dependent upon the incubation temperature and perhaps stock selected for the experiment.

Materials and Methods

We collected gametes from 15 male and female chum salmon from the Nitinat River on the west coast of Vancouver Island on October 31, 1983. Postorbital–hypural length of females was measured to the nearest millimetre and scale samples were obtained for subsequent age determination. Sperm from a single male fertilized the eggs of a single female, and after water-hardening, 30 eggs from each family were preserved in 10% formalin for at least 90 d for subsequent determination of egg size. The diameter of an egg was measured with calipers along the longest axis to the nearest 0.1 mm. Egg weight (milligrams) was obtained by first blotting the egg to remove excess surface moisture, and then weighing the egg on a top-loading electrobalance.

Eggs were incubated in vertical stack incubators, with each tray partitioned into 20 compartments with a plastic divider. Three egg lots from each family were assigned to each of three stacks, with each replicate in a different tray in each stack. The trays were kept in darkness and provided with a flow of 6.9 L/min of dechlorinated Nanaimo tap water. Target incubation temperatures of 4, 8, and 12°C were set for each of three stacks, respectively, and observed temperatures (and standard deviations) from egg fertilization to hatching were 4.0 (0.23), 8.0 (0.18), and 12.0°C (0.16), respectively. Mean water temperatures from fertilization to emergence of the fry were 4.0 (0.21), 8.0 (0.20), and 12.0°C (0.19), respectively. Water temperatures in each stack were recorded daily. Oxygen concentrations were above 90% of air saturation at each temperature.

Dead eggs were removed from each compartment and stored in Stockard's solution (Rugh 1952). All dead eggs were inspected to determine which were unfertilized or the stage of development at which they died. We recognized three stages of development: fertilization to epiboly (stage 17 of Vernier 1969), epiboly to eye pigmentation (stage 25), and eye pigmentation to hatching (stage 30). Within 1 d of 50% hatching, 10 alevins from each compartment (30/family) were preserved in 10% formalin for subsequent determination of length and weights. After hatching, the alevins were provided with a gravel substrate, with particle size 2–3 cm in diameter. After preservation for 90 d, alevins were blotted to remove excess surface moisture, and total (fork) length was measured to the nearest 0.1 mm. Total weight of the alevin was also recorded. The yolk was then separated from the rest of the body tissue and weighed. Tissue weight was then determined by subtraction. Dead alevins were removed from each compartment and the number recorded to determine survival rates from hatching to fry emergence.

We used an emergence trap (modified from Godin 1980) to determine timing of fry emergence. We constructed the trap from a Plexiglas aquarium bottom filter complete with lid and standpipe by placing a 4-cm layer of gravel in the bottom of the filter and fitting a layer of reinforced black plastic cut to fit over the standpipe and the gravel. A 6-mm hole was cut in the plastic to enable fry to move out of the gravel when they emerged. These traps, each with its own air supply, were placed in 250-L tanks of water at the appropriate temperature. Thirty alevins from each family (10/replicate) were placed in the gravel when they were about two thirds "buttoned-up" (late Vernier stage 35). The emergence traps were exposed to fluorescent lighting during the working day and natural light during the remainder of the day. The emergence traps were checked each afternoon for emergent fry. When 50% of the fry for a particular family had emerged, the remainder of the family in the stack was preserved in 10% formalin. After preservation for 90 d, 14 fry/replicate were unselectively drawn from the preserved sample and fry fork length, total weight, yolk weight, and tissue weight determined as for alevins.

Females were grouped into three size classes based upon their postorbital–hypural length (Table 1). Ages were determined from scales. Heterogeneity of egg and alevin survival rates among size classes and among families within classes was tested by likelihood ratio (G-statistic) (Sokal and Rohlf 1969). Equality of alevin and fry lengths and weights among the replicates was tested by doubly nested analysis of variance using the following model (temperatures separate):

$$Y_{ijkl} = S_i + F_{ij} + R_{ijk} + e_{ijkl}$$

where Y = length or weight, S = size class (i = 1 and 3), F = family within size class (j = 1 and 5), R = replicate within family (k = 1 and 3), and e = error term for the lth observation in subgroup ijk. All effects were considered random. Egg size differences among size classes and among families within classes were tested by a similar model without the replicate term. Equality of alevin and fry lengths and weights among temperatures, size classes, and families was tested with the following model (all effects random):

$$Y_{ijkl} = T_i + S_j + TS_{ij} + F_{jk} + e_{ijkl}$$

where Y = length or weight, T = temperature (i = 1 and 3), S = size class (j = 1 and 3), TS = temperature and stock interaction, F = family within stock (k = 1 and 5), and e = error term for the lth observation in subgroup ijk.

Results

Female and Egg Size

Females were grouped into three size classes based upon their postorbital–hypural length ("large," "medium," and "small"), and there were significant differences in length among the classes ($F = 43.99$, df = 2,12, $P < 0.01$) (Table 1). Although there was significant variation in egg diameter among families within size classes ($F = 16.27$, df = 12,435, $P < 0.01$), there was no significant difference among size classes relative to variation within the classes ($F = 1.95$, df = 2,12, $P > 0.05$). There were significant differences in egg weight both among families within size classes ($F = 31.98$, $P < 0.01$) and among classes ($F = 7.19$, $P < 0.05$). Females in the small size class had lighter eggs than those in the larger size classes.

Egg diameter and egg weight were dependent upon female body length. As female body length increased, egg diameter also increased ($r = 0.56$, df = 13, $P < 0.05$), as did egg weight ($r = 0.65$, $P < 0.01$), but more of the variance in egg weight was accounted for by female length than by egg diameter. Subsequent analysis of variation resulting from egg size differences is based solely on egg weights.

TABLE 1. Postorbital–hypural length, age, egg diameter, and egg weight of 15 Nitinat River chum salmon females. Females were divided into three size classes. Standard deviations are given in parentheses. Thirty eggs were measured and weighed for each female.

Class	Female	Length (mm)	Age (yr)	Egg diameter (mm)	Egg weight (mg)
Large	1	605	4	8.2(0.2)	303.7(12.1)
	2	600	4	8.6(0.3)	348.9(19.8)
	3	640	4	8.4(0.2)	315.8(14.6)
	4	594	4	8.2(0.2)	300.0(7.3)
	5	623	4	8.5(0.2)	314.4(9.5)
	Mean	612.4	4.0	8.4(0.2)	316.6(12.7)
Medium	1	572	4	8.4(0.4)	332.7(47.0)
	2	565	4	8.6(0.4)	368.6(47.7)
	3	540	3	8.1(0.3)	284.0(31.5)
	4	538	3	8.3(0.2)	282.1(21.7)
	5	534	3	7.9(0.2)	283.8(27.9)
	Mean	549.8	3.6	8.3(0.3)	310.2(35.2)
Small	1	502	3	8.2(0.3)	275.8(23.1)
	2	525	3	7.9(0.3)	230.2(29.0)
	3	532	3	8.2(0.3)	268.5(24.4)
	4	510	3	8.0(0.3)	263.8(19.2)
	5	517	3	8.4(0.3)	246.7(22.5)
	Mean	517.2	3.0	8.1(0.3)	257.0(23.6)

Survival

Egg survival from fertilization to hatching was highest for eggs from small-sized females and lowest for eggs from large-sized females at all three incubation temperatures (Table 2). Egg densities also increased from the small to the large size class, but egg mortality rates were not related to initial loading densities. For example, within a size class, there was no relation between family loading density and subsequent family survival within each temperature (12°C, large $r = 0.19$, medium $r = 0.45$, small $r = 0.84$; 8°C, large $r = 0.92$, medium $r = 0.79$, small $r = 0.68$; 4°C, large $r = 0.12$, medium $r = 0.32$, small $r = 0.81$). In fact, all correlations between loading density and subsequent survival of families within size classes were all positive, which suggests that differences in egg survival rates among the different size classes were not dependent upon initial egg density. Eggs from large females were less viable than those from smaller ones.

Incubation temperature had a marked effect on egg survival. Egg survival was lower at 12 and 4°C than at 8°C for all three size classes (Tables 2, 3). At 8°C, egg survival rates of all three size classes were at least 96%. Differences in survival rates at 8°C and those at 4 and 12°C became larger as female size increased. For example, eggs from small females had 88% survival rates at 12°C and 97% at 8°C, a differential of 9%, whereas eggs from large females had survival rates of 77 and 96%, respectively, a differential of 19% (Table 2). Eggs from large females were more sensitive to temperature extremes than were eggs from small females.

Egg survival at 4°C was largely determined by survival rates from fertilization to epiboly. Egg mortality at this stage was higher at 4°C than at higher incubation temperatures (Table 2). At 12°C, the lowest egg survival rates were recorded from eye pigmentation to hatching for eggs from all three size classes of females. The stage of development where most mortality occurs during incubation is clearly dependent upon temperature.

Variability in egg survival rates among families within each size class was greatest at 4 and 12°C (Fig. 1; Table 4). Although there were significant differences in egg survival rates among the size classes at each temperature, heterogeneity in survival rates among families within each class was greater than heterogeneity among the size classes at each temperature (Table 4). Survival rates among families within a size class could vary from 45 to 100% at a given temperature (Fig. 1). Family variation in egg survival rates could be considerable, and was accentuated as temperatures became more extreme.

At 8 and 12°C, alevin survival in the small size class was always higher than those in the two larger classes (Table 2), but the differences between pairs of classes were not always significant (Table 4). Alevin survival increased as incubation temperature declined, with 100% alevin survival observed at 4°C in all 15 families examined. The alevins that died at 12°C died a few days after hatching and were characterized by sluggish movements and a fungus infection in the gills shortly before death.

As with eggs, variability in alevin survival rates among families within size classes was greater than variability among the size classes (Table 4; Fig. 1). Alevins from some families

TABLE 2. Survival rates of eggs and alevins for three size classes of Nitinat River chum salmon females incubated at controlled water temperatures of 4, 8, and 12°C. Times of 50% hatching of the alevins and 50% emergence for the fry are indicated. *N* is number of fertilized eggs (for egg survival rates) or alevins hatching (for alevin survival rates). Standard deviations of hatching and emergence time are given in parentheses.

		Eggs						Alevins			
Class	Temperature (°C)	*N*	Fertilization to epiboly	Epiboly to eyed	Eyed to hatch	Fertilization to hatch	Time to 50% hatch (d)	*N*	Hatch to emergence	Fertilization to emergence	Time to 50% emergence (d)
Large	4	3608	0.83	0.93	0.96	0.74	124.5(0.5)	2671	1.00	0.74	193.9(2.1)
	8	2984	0.99	0.99	0.99	0.96	67.5(0.5)	2872	0.97	0.94	108.5(1.1)
	12	3496	0.93	0.93	0.89	0.77	44.8(0.8)	2687	0.69	0.53	77.7(1.3)
Medium	4	3249	0.85	0.99	0.98	0.82	124.7(1.2)	2659	1.00	0.82	195.0(2.1)
	8	2416	0.97	0.99	0.99	0.96	67.3(0.7)	2320	0.98	0.94	108.2(1.8)
	12	2597	0.91	0.98	0.90	0.81	45.2(0.5)	2097	0.72	0.58	77.4(1.9)
Small	4	2530	0.87	0.99	0.98	0.84	123.6(1.5)	2137	1.00	0.84	193.2(1.1)
	8	2298	0.99	0.99	0.99	0.97	66.6(1.0)	2237	0.99	0.96	107.0(1.4)
	12	2302	0.96	0.99	0.93	0.88	45.1(0.4)	2022	0.76	0.67	75.9(1.4)

TABLE 3. Likelihood ratio (G-test) for heterogeneity of survival rates of eggs and alevins from three size classes of Nitinat River female chum salmon. Eggs and alevins were incubated at controlled temperatures of 4, 8, and 12°C. *$P < 0.05$.

	Eggs		Alevins	
	df	G	df	G
12°C vs. 8°C	3	1023.2*	3	2207.2*
Large	1	558.6*	1	929.1*
Medium	1	303.0*	1	690.5*
Small	1	161.6*	1	587.6*
12°C vs. 4°C	3	35.9*	3	3115.5*
Large	1	23.6*	1	1286.3*
Medium	1	1.1	1	1064.6*
Small	1	11.2*	1	764.6*
8°C vs. 4°C	3	906.4*	3	192.7*
Large	1	352.1*	1	89.6*
Medium	1	294.0*	1	65.1*
Small	1	260.3*	1	38.0*

survived much better than did others, and the survival rates were not related to initial alevin density. Overall survival rates from egg fertilization to fry emergence were most divergent between the small and large size classes at 12°C.

Hatching and Emergence Time

Eggs derived from females of different length all hatched at the same time when groups incubated at the same water temperatures were compared (Table 2) (all $P > 0.05$). Emergence timing of the fry was also similar among the three size classes at each temperature (all $P > 0.05$). In the Nitinat River stock, eggs fertilized at the same time and subjected to the same incubation temperatures produce fry that emerge at the same time from the emergence traps. As expected, hatching and emergence time increased with declining water temperature.

Alevin Size

No significant differences in alevin or fry length and weight were detected among the replicates for individual females, and the replicates were combined for further analysis. Alevins hatching at 4°C were the longest and those at 12°C were the shortest (Table 5; Fig. 2) ($F = 60.75$, df = 2,4, $P < 0.01$). Alevins derived from large females were longer than those from small ones ($F = 4.75$, df = 2,16, $P < 0.05$) (Table 5). There were significant differences in alevin length among families within size classes ($F = 13.51$, df = 12,1314, $P < 0.01$).

Egg weight was shown previously to be significantly related to female body length (Table 1), and we examined the relationship between egg weight and alevin length at each incubation temperature. Although all three correlations were positive, the only one approaching significance was at 12°C ($r = 0.45$, df = 13, $0.05 < P < 0.10$) (Fig. 3). The effect of egg size on subsequent alevin length was dependent upon the incubation regime used.

The total weight of alevins at hatching was dependent upon both incubation temperature ($F = 23.21$, $P < 0.01$) and female length ($F = 129.73$, $P < 0.01$) (Table 5). Given the variation in egg weight among families within size classes (Table 1), there were also significant differences in total weights of alevins among families within classes ($F = 86.50$, $P < 0.01$). Total weight of alevins declined as incubation temperature declined and large females produced heavier alevins than did small ones.

The total weight of an alevin may be partitioned into the weight of the yolk and that of the body tissue. Although there were significant differences in total alevin weight among incubation temperatures, with the heaviest alevins produced at 12°C, there were no differences in alevin yolk reserves at hatching within a size class attributable to incubation temperature ($F = 5.30$, $P > 0.05$) (Table 5). There were, however, clear differences in alevin yolk reserves attributable to female size ($F = 100.59$, $P < 0.01$) and variation among families within size class ($F = 180.51$, $P < 0.01$). Large females produced alevins with greater yolk reserves.

As there were significant differences in total alevin weight among incubation temperatures, but not yolk weight, there must be differences in alevin tissue weight ($F = 74.81$, $P < 0.01$). Within each size class, alevins with the heaviest tissue weight were produced at 12°C, and the lightest at 4°C (Table 5). Alevins from large females also had greater tissue weights at each temperature than did those from small females ($F = 78.32$, $P < 0.01$). Alevin tissue weight at hatching is clearly influenced by the efficiency of conversion of yolk to body tissue at the different incubation temperatures.

Alevin tissue weight at hatching was positively correlated with initial egg weight at all three incubation temperatures, but

TABLE 4. Likelihood ratio (G-test) for heterogeneity of survival rates of eggs and alevins from three size classes of Nitinat River female chum salmon. Eggs and alevins were incubated at controlled temperatures of 4, 8, and 12°C. *$P < 0.05$.

	12°C				8°C				4°C				Total				Standardized measure	
	Egg		Alevin		Egg		Alevin		Egg		Alevin		Egg		Alevin			
	df	G	df	G	df	G	df	G	df	G	df	G	df	G	df	G	Egg	Alevin
Among sizes	2	115.0*	2	25.9*	2	7.4*	2	7.6*	2	9.2*	2	0.0	6	131.6*	6	33.5*	21.9	5.6
Within sizes	12	1531.6*	12	426.3*	12	125.4*	12	173.0*	12	1021.7	12	0.0	36	2678.7*	36	599.3*	74.4	16.6
Large	4	386.2*	4	241.3*	4	31.3*	4	132.2*	4	408.5*	4	0.0	12	826.0*	12	373.5*	68.8	31.1
Medium	4	692.0*	4	86.3*	4	84.9*	4	30.2*	4	350.9*	4	0.0	12	1127.8*	12	116.5*	94.0	9.7
Small	4	453.4*	4	98.7*	4	9.2*	4	10.6*	4	262.3*	4	0.0	12	724.9*	12	109.3*	60.4	9.1
Large vs. medium	1	13.2*	1	4.3*	1	0.1	1	1.4	1	60.3*	1	0.0	3	13.3*	3	5.7	4.4	1.9
Large vs. small	1	114.0*	1	25.6*	1	4.7*	1	7.0*	1	97.6*	1	0.0	3	126.0*	3	32.6*	42.0	10.9
Medium vs. small	1	46.0*	1	7.9*	1	6.0*	1	1.8	1	6.8*	1	0.0	3	58.8*	3	9.8*	19.6	3.3
Total	14	1646.6*	14	452.2*	14	132.8*	14	180.6*	14	1030.9*	14	0.0	42	2810.3*	42	632.8*	66.8	15.1

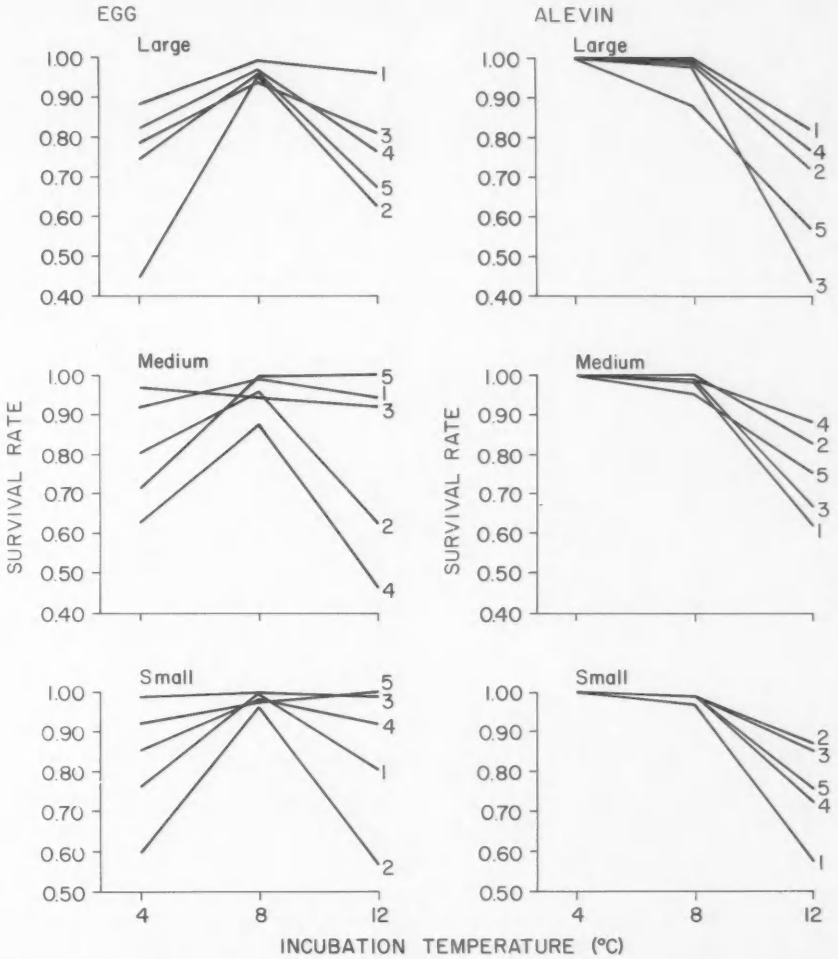

FIG. 1. Survival rates of eggs from fertilization to hatching and of alevins from hatching to emergence for five chum salmon families (numbered 1–5) in each of three female size classes at incubation temperatures of 4, 8, and 12°C.

TABLE 5. Mean fork length, total weight, yolk weight, and tissue weight for alevins and fry from three size classes of Nitinat River female chum salmon. Eggs and alevins were incubated at controlled temperatures of 4, 8, and 12°C. *N* is sample size. Standard deviations are given in parentheses.

		Alevins					Fry				
Class	Temperature (°C)	*N*	Fork length (mm)	Total weight (mg)	Yolk weight (mg)	Tissue weight (mg)	*N*	Fork length (mm)	Total weight (mg)	Yolk weight (mg)	Tissue weight (mg)
Large	4	150	24.3(0.9)	313.9(25.7)	197.8(16.2)	116.1(17.3)	210	37.6(1.0)	425.1(42.4)	40.8(14.8)	384.3(28.0)
	8	150	23.9(0.7)	330.5(23.7)	202.2(15.0)	128.2(17.5)	210	37.2(1.2)	422.9(31.3)	51.2(12.2)	371.8(31.3)
	12	147	22.7(0.7)	337.6(32.9)	204.2(20.4)	133.4(18.9)	210	36.8(1.1)	462.6(44.4)	50.6(10.8)	412.1(44.0)
Medium	4	150	23.6(0.7)	287.1(24.8)	183.3(17.0)	103.8(15.1)	210	36.8(1.0)	392.2(35.2)	41.4(12.1)	350.8(28.9)
	8	150	23.4(0.6)	293.1(25.1)	178.1(21.5)	115.0(25.5)	209	36.3(1.2)	380.2(31.4)	42.1(16.6)	338.1(31.2)
	12	144	22.4(0.7)	309.3(26.1)	189.7(20.6)	119.6(15.8)	203	25.0(1.7)	406.0(51.4)	48.9(16.2)	357.1(55.6)
Small	4	149	23.3(1.0)	270.6(15.8)	165.3(13.8)	105.3(11.5)	210	36.1(0.9)	373.4(36.7)	33.4(18.3)	340.0(36.1)
	8	148	23.4(0.7)	273.2(21.7)	160.7(16.2)	112.5(14.8)	210	36.1(0.8)	356.6(25.7)	32.3(10.9)	324.3(22.2)
	12	147	22.2(0.9)	288.5(22.2)	171.0(13.9)	117.4(12.5)	194	34.8(1.3)	375.2(36.4)	41.9(12.7)	333.2(42.0)

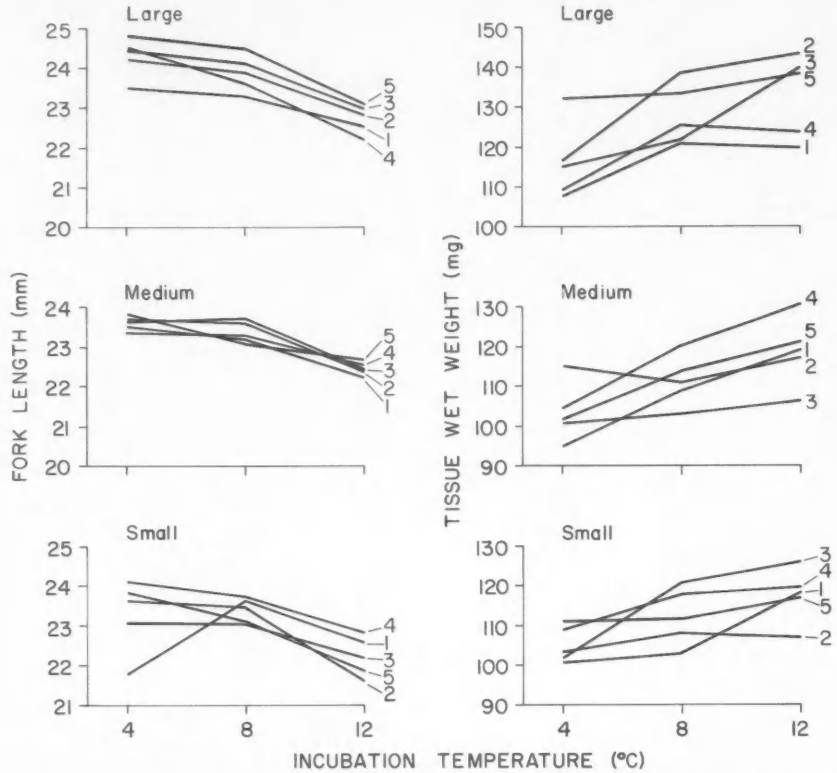

FIG. 2. Fork length and tissue weight of alevins at hatching for five Nitinat River chum salmon families in each of 3 female size classes at incubation temperatures of 4, 8, and 12°C.

the correlation approached significance only at 12°C ($r = 0.47$, df = 13, $0.05 < P < 0.10$) (Fig. 4). As the previous analysis suggested, whether or not heavy tissue weight alevins are derived from large eggs is dependent upon incubation temperature and the efficiency of yolk conversion at the temperatures investigated. If the incubation temperatures are in the range where a stock has a lowered conversion efficiency of yolk to body tissue, then the relationship between egg size and alevin weight will become less pronounced.

Nitinat River chum salmon alevins were the shortest at 12°C, but they also had the heaviest tissue weight and total weight at 12°C (Fig. 2; Table 5). These two factors combine to produce alevins with the highest index of development (K_D, Bams 1970) values (K_D = 10·alevin or fry weight$^{0.333}$/alevin or fry length) (Fig. 5). The K_D values differed both among incubation temperatures, being highest at 12°C ($F = 66.30$, $P < 0.01$), and among size classes, being greatest in alevins from large females ($F = 6.10$, $P < 0.05$), as well as among families within classes ($F = 26.59$, $P < 0.01$). Within a size class, alevins derived from heavier eggs usually had greater K_D values than those derived from lighter ones (Fig. 5). The K_D values are clearly influenced by both initial egg size and incubation temperatures.

Fry Size

Length of emergent fry declined as incubation temperature increased ($F = 12.29$, df = 2,4, $P < 0.05$ (Fig. 6) and as female length declined ($F = 5.17$, df = 2,16, $P < 0.05$) (Table 5). Fry with the greatest tissue weight were produced at 12°C. As with the alevins, there were significant differences in fry length among families within size classes ($F = 45.63$, df = 12,1865, $P < 0.01$). Relationships between egg weight and resultant fry length were stronger for fry than for alevins. There were significant correlations at 4°C ($r = 0.80$, df = 13, $P < 0.01$) and 8°C ($r = 0.69$, $P < 0.01$), but not at 12°C (Fig. 3). Some of the alevins from different families developed elongated and coagulated yolk sacs (Emadi 1973) at 12°C, and this could account for the lack of a relationship between fry length and egg weight at 12°C when there was a relationship between alevin length and egg weight at 12°C. Although there was no significant relationship between alevin length and egg weight at 4 or 8°C, the relationship was well established for fry at emergence (Fig. 3). Longer fry were produced from heavier eggs.

As with alevins, the total weight of fry was dependent upon incubation temperature ($F = 7.79$, $P < 0.05$) and female size ($F = 43.25$, $P < 0.05$), with the heaviest fry produced at 12°C and from large females (Table 5). There were no differences in fry yolk reserves within a size class at emergence at the different incubation temperatures ($F = 5.01$, $P > 0.05$), but fry from large females had the greatest yolk reserves and fry from small females had the least ($F = 9.36$, $P < 0.05$) (Table 5).

There were no significant differences in fry tissue weight at the different incubation temperatures ($F = 4.56$, $P > 0.05$), but large females produced heavier fry than did small females ($F = 27.58$, $P < 0.01$). Fry tissue weight was significantly correlated

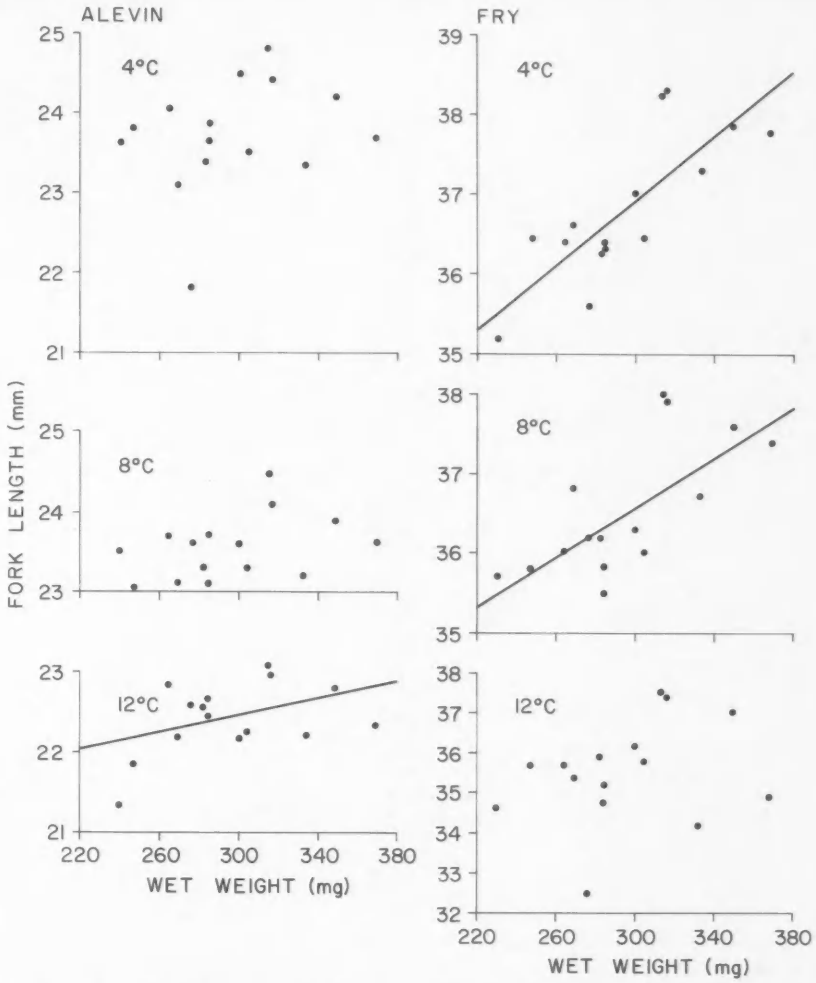

FIG. 3. Relationship between alevin and fry fork length and egg weight for 15 families of Nitinat River chum salmon incubated under controlled temperatures of 4, 8, and 12°C.

with initial egg size at 4°C ($r = 0.65$, $P < 0.01$) and 8°C ($r = 0.64$, $P < 0.01$) and approached significance at 12°C ($r = 0.45$, $0.05 < P < 0.10$) (Fig. 4). Heavier fry are derived from larger eggs at all three incubation temperatures.

After hatching, the relative proportions of alevin yolk and body tissue may provide an indication of potential fry tissue weight. We assumed that yolk was 50% water and that tissue was 84% water (Heming 1982) and compared the actual growth of fry relative to the potential of the alevins. Alevins with higher proportions of yolk relative to body tissue at hatching had higher proportions of body tissue as fry relative to their tissue weight as alevins ($r = 0.85$, df = 7, $P < 0.01$) (Fig. 7). In each female size class, alevins hatching at 4°C had less tissue weight but similar yolk weights than those hatching at 12°C. The alevins hatching at the lower temperatures used their greater amounts of yolk relative to body tissue for higher growth rates before emergence than did alevins hatching at higher temperature, with the result that at emergence, fry tissue weights within a size class were similar at the three incubation temperatures.

Like alevins, Nitinat River fry were the shortest but heaviest at 12°C (Fig. 6), with the result that K_D values were highest at 12°C ($F = 40.45$, $P < 0.01$) (Fig. 5). Families that produced alevins with higher K_D values also tended to produce fry with higher values, even though there were no differences in hatching time of the alevins or emergence of the fry among the size groups.

Discussion

One objective of our study was to examine egg survival in relation to female body length. Previous investigations indicated that survival rates of eggs during incubation from small females were higher than those from large females for coho salmon (*Oncorhynchus kisutch*) (Allen 1957), chinook salmon (*O. tshawytscha*) (Fowler 1972), and rainbow trout (*Salmo gairdneri*) (Murray 1980). Fecundity of chum salmon increases as body length increases (Bakkala 1970; Beacham 1982) as does egg size (Watanabe 1955; this study). If survival rates for all fish

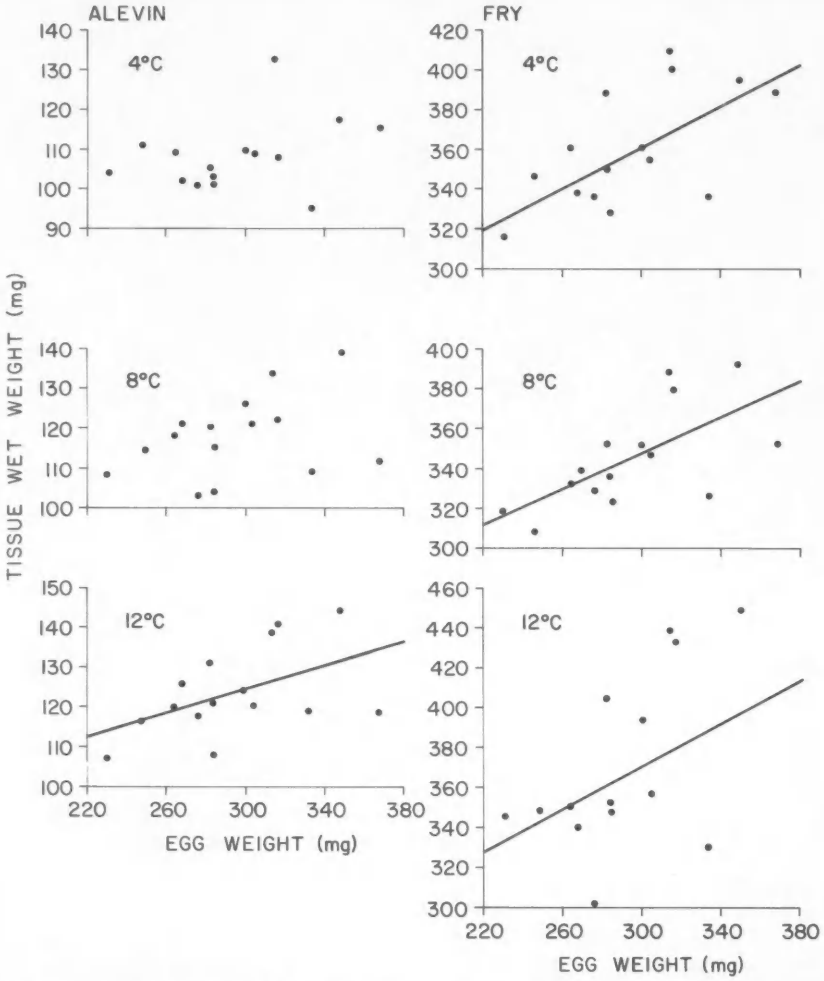

FIG. 4. Relationship between alevin and fry tissue weight and egg weight for 15 families of Nitinat River chum salmon incubated under controlled temperatures of 4, 8, and 12°C.

were equal from egg fertilization to adult return, and adults all returned at the same age, then selection would favour females of large body size and high fecundity rather than small females. However, large chum salmon mature at older ages than smaller ones, and increased fecundity of larger females compensates for the additional year or two of ocean mortality in their life history. If the mean length of age 3 and age 4 Nitinat River female chum salmon is estimated at 539 and 586 mm, respectively (McCart et al. 1980), the mean fecundities are 2385 and 2826 eggs, respectively (Beacham 1982), with age 4 females 19% more fecund than age 3 females. Instantaneous rates of natural mortality during the final year of ocean residence are estimated at 0.015/mo, or about 20%/yr (Ricker 1976), so that additional mortality during the final year of ocean life of an age 4 female is compensated for by increased fecundity. Older larger females produce larger eggs and fry than younger smaller ones, and if large fry have higher survival rates than do small fry (Wallace and Aasjord 1984), decreased freshwater survival rates of large eggs may be compensated for by increased early marine survival or large fry.

Eggs from large females survived less well than those from small females at both 4 and 12°C. At 4°C, most of the mortality occurred during fertilization to epiboly. The duration of epiboly in salmonids may be influenced by the ratio of yolk to cytoplasm in the egg, with eggs having relatively greater amounts of yolk taking longer to complete epiboly (Smirnov 1975). Increased egg mortality during salmonid embryonic development occurs during epiboly (Battle 1944), and higher rates of mortality of the larger eggs may be partially accounted for by the increased duration of epiboly. Higher mortalities of large eggs at 12°C may be partially attributable to insufficient oxygen in the water, even though oxygen concentrations were always at least 90% of saturation levels. Larger eggs have a smaller surface to volume ratio than smaller eggs, and because the oxygen level at the egg

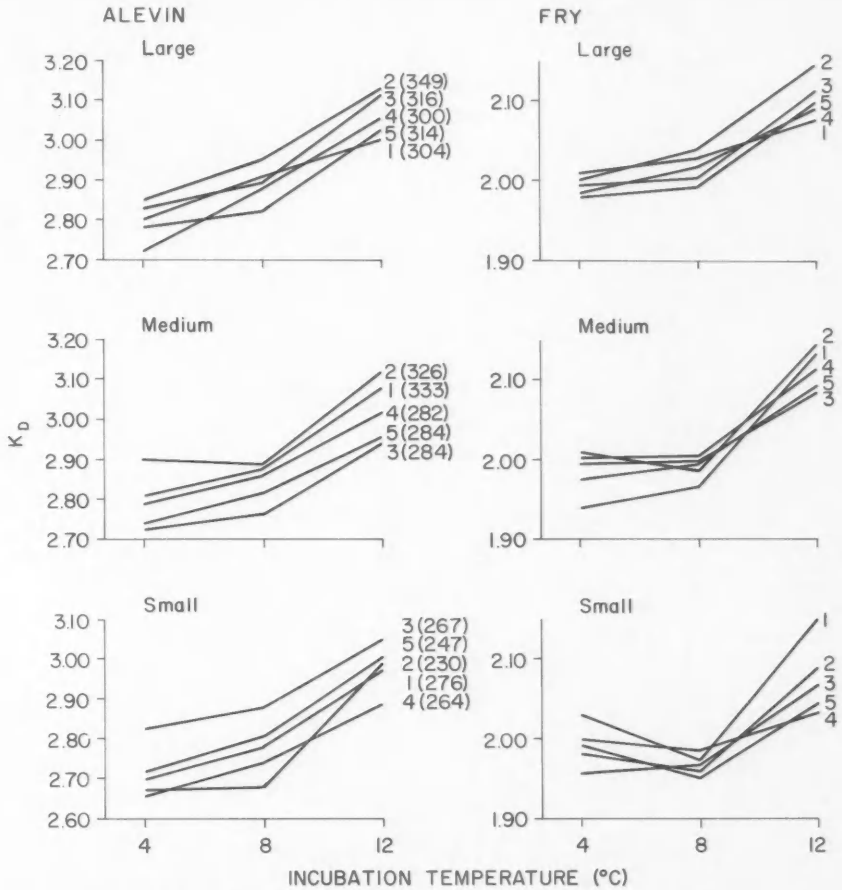

FIG. 5. K_D values (Bams 1970) from alevins at 50% hatch and fry at 50% emergence for five chum salmon families in each of three female size classes. Egg weights (mg) are indicated in parentheses.

surface may be much less than that of the water supply even at high water velocities (Daykin 1965), oxygen deficiencies may result in increased mortality of large eggs.

Large eggs have lower survival rates than small eggs. Why, then, do large females not produce eggs of the same size as small females? Two aspects of salmonid developmental biology dependent upon egg size may be hatching and emergence times of the alevins and fry and their size at hatching and emergence. Hatching and emergence timing of Nitinat River chum salmon alevins and fry were independent of egg size when the same incubation temperature was examined. Fry from large eggs had, however, greater yolk reserves at emergence than did fry from small eggs. This greater yolk reserve may confer a survival advantage to fry from large eggs if marine conditions are unsuitable, as demonstrated for herring (*Clupea harengus*) by Blaxter and Hempel (1963).

Size of salmonid fry may be a significant determinant of subsequent growth and survival (Bams 1969; Fowler 1972), with larger fry having higher survival rates. Nitinat River chum salmon fry length at emergence was greater for fry from large females than from small ones, so that fry from large females should have higher survival rates than those from smaller ones, presumably because they are better able to avoid predators during early marine life (Healey 1982). Lower survival rates of large eggs during incubation are negated by higher survival rates of the fry during early marine residence.

Bams (1970) developed an index (K_D) to compare the fork lengths and weights of salmonid fry to determine if they had emerged from the gravel at the same stage of development. Heming (1982) interpreted the shorter lengths and higher K_D values of chinook salmon alevins incubated at 12°C to be indicative of premature hatching at that temperature. In our study, Nitinat River chum salmon alevins hatching at 12°C were the shortest, had the highest K_D values, but were also the heaviest. The high K_D values for alevins hatching at 12°C cannot be interpreted as evidence for premature hatching, as they have the highest tissue weights of all the alevins in our study. Rather than interpreting K_D as a developmental index, we suggest that is more properly interpreted as a condition factor.

Larger alevins and fry have been reported to be derived from larger salmonid eggs (Smirnov 1975; Kazakov 1981; Wallace and Aasjord 1984), but no difference in alevin size has also been reported for alevins derived from large and small eggs

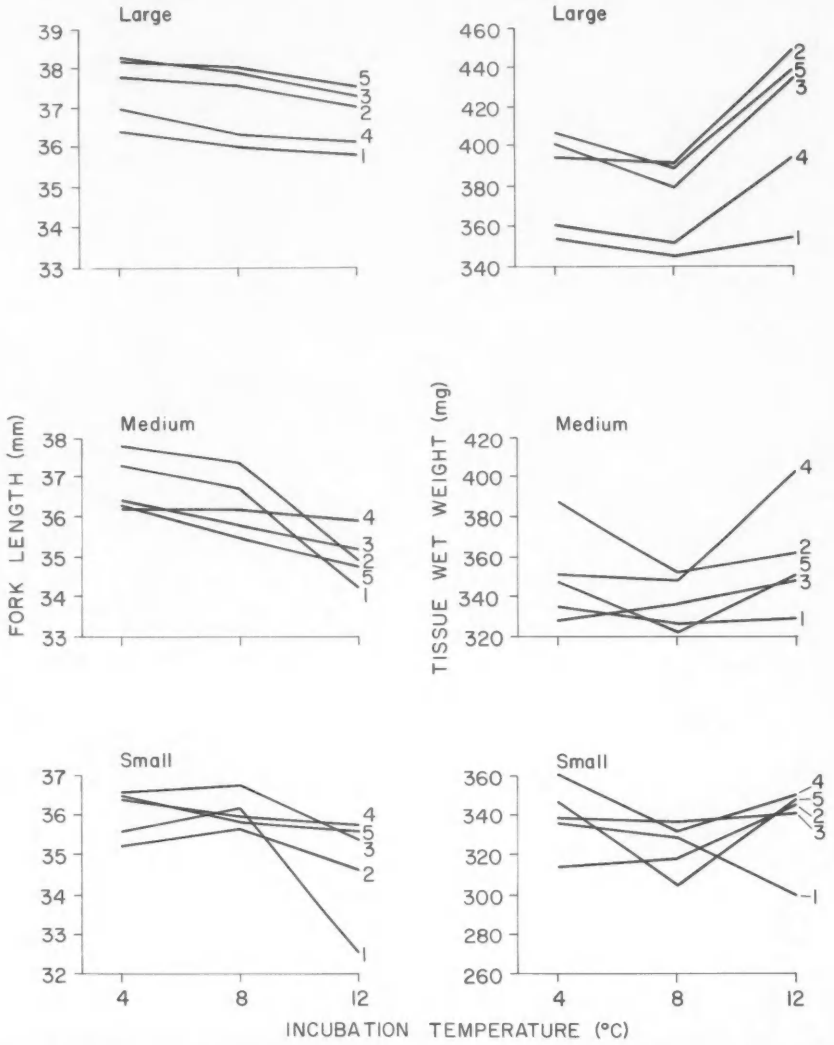

FIG. 6. Fork length and tissue weight of fry at emergence for five Nitinat River chum salmon families in each of three female size classes at incubation temperatures of 4, 8, and 12°C.

(Privol'nev et al. 1964; Yastrebkov 1966; Beacham et al. 1985). Both results were observed in our study of Nitinat River chum salmon. As incubation temperature increased, alevins at hatching had converted more of their yolk reserves to body tissue, and thus a relationship between egg weight and alevin tissue weight only became apparent at temperatures in the range of optimum yolk conversion efficiency. Whether or not an effect of egg size on alevin size can be demonstrated depends upon the temperature regime used in the experiment and the temperature of optimum yolk conversion efficiency for the stock under investigation. For example, Beacham et al. (1985) could demonstrate no effect of egg size on alevin tissue weight on Big Qualicum River chum salmon, a stock on the east coast of Vancouver Island, but the eggs were incubated at a mean temperature of 4.8°C. However, this result might be expected if the temperature of optimum yolk conversion efficiency of the Big Qualicum River stock is near 12°C, similar to that of the Nitinat River stock, situated on the west coast of Vancouver Island. The effect of egg size on subsequent alevin and fry size is dependent upon incubation temperature.

In our study, there were significant differences in alevin tissue weights at hatching for the different incubation temperatures, but at the time of fry emergence, no significant differences in tissue weight with respect to incubation temperature could be demonstrated. Alevins hatching at 4°C had lower tissue weights than those hatching at 12°C, but they grew faster after hatching than did the 12°C alevins, so that fry tissue weights under the three temperature regimes were similar at emergence.

As most marine mortality is thought to occur during early marine residence (Parker 1968), and mortality is size selective (Healey 1982), initial egg size may be a significant factor in population dynamics of a salmon stock by influencing the length

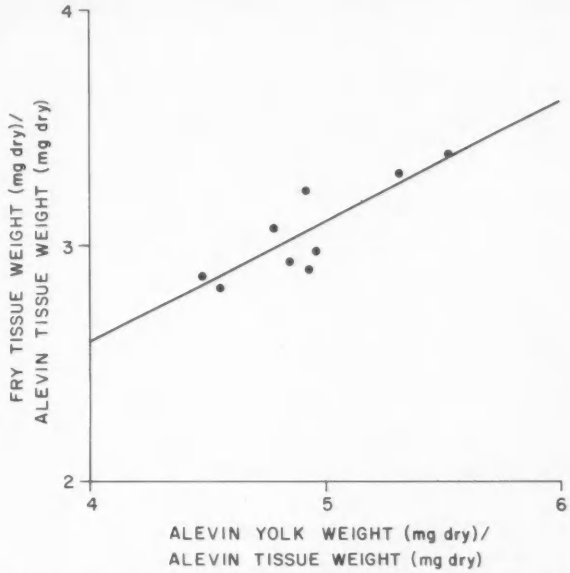

FIG. 7. Relationship between the relative proportions of fry and alevin tissue weight and alevin yolk and tissue weight.

of emergent fry. Variations in stock developmental biology in response to changing environmental conditions may show adaptations of stocks to their spawning environments.

Acknowledgments

Arthur Demsky, Eric Lauinger, Tim Netherall, and Karen Shillington weighed and measured the chum salmon eggs, alevins, and fry examined in this study and also determined the stage of development of the eggs that died. Bruce Bernard was of exceptional assistance in most of the data analysis. Lauri Mackie drafted the figures. The assistance of the staff at the Nitinat River hatchery is also greatly appreciated. Drs. S. Schroeder and J. C. Wallace provided many helpful suggestions to improve the manuscript.

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Equilibrium Yields and Yield Isopleths from a General Age-Structured Model of Harvested Populations

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The equilibrium properties of an age-structured model that includes any arbitrary age-specific weights, vulnerabilities, fecundities, and natural mortality rates, combined with stock–recruitment relationships, are derived. The numbers, biomass, and catch at each age can be calculated quite simply. These relationships can be used to construct yield-isopleth diagrams, or to plot equilibrium yield and biomass against harvest intensity. We used the results to compute yield isopleths for the Pacific halibut (*Hippoglossus stenolepis*) fishery. The analysis can also include a fishing season of any specified length. Relationships are given to translate the aggregate properties of the age-structured models into several alternative surplus production models.

On a calculé les propriétés d'équilibre d'un modèle structuré selon l'âge qui comprend différents poids, vulnérabilités, fécondités et taux de mortalité naturelle arbitraires en fonction de l'âge combinés à des relations stock–recruitment. Les nombres, biomasses et prises selon l'âge peuvent être facilement calculés. Ces relations peuvent servir à l'élaboration de diagrammes des lignes isoplèthes du rendement ou à tracer un graphique du rendement d'équilibre et de la biomasse en fonction du niveau d'exploitation. Les auteurs ont utilisé les résultats pour calculer les lignes isoplèthes du rendement de la pêche du flétan du Pacifique (*Hippoglossus stenolepis*). L'analyse peut aussi porter sur une saison de pêche de diverse durée. On présente des relations pour transformer les propriétés globales des modèles structurés selon l'âge en plusieurs autres modèles de production excédentaire.

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Age-structured models of fish stocks are increasingly used in the analysis of fisheries management options; such models follow the general form outlined by Walters (1969). These models can include age-specific natural mortality rates, fecundity, weight, and vulnerability to fishing, as well as stock–recruitment relationships. Normal practice is to use such models in simulations of alternative fishing policies, although a literature has developed on the optimal harvesting strategies for these models: Rorres and Fair (1975), Reed (1980), Getz (1980a, 1980b). Fisheries managers often want to explore the relationship between harvest intensity and some aspect of age-specific vulnerability, a method popularized by the yield-isopleth diagrams of Beverton and Holt (1957). Beverton and Holt's work did not include any stock-recruitment relationships and therefore is often inadequate for stocks when stock and recruitment are thought to be related.

In this paper we explore the properties of a generalized age-structured model for generation of yield-isopleth diagrams, and we present a worked example using data from Pacific halibut (*Hippoglossus stenolepis*). The equations we derive are closely related to those of Getz (1980a) who analyzed an age-structured model that differs primarily in the timing of the breeding season in relation to the harvest season.

In addition we explore a number of properties of the model to see if biologically meaningful parameters for stock and recruitment can be derived. Finally we see to what extent the properties of the generalized age-structured model can be compressed into simple two-parameter surplus production models.

Reconsideration of the Catch Equation

Since the catch equation was introduced over 60 yr ago (Baranov 1918), methods of fish stock assessment have assumed that fishing mortality and mortality due to causes other than fishing act simultaneously over the course of the entire year. However, the fishing season for most stocks has been greatly reduced since Baranov's day and it is therefore often assumed that fishing and natural mortality occur separately (e.g. the method of Deriso 1980). Both assumptions are extreme. An intermediate model, in which fishing takes place over a proportion of each year but where natural mortality takes place over the entire year, is shown below.

If we let our year begin with the onset of fishing and if the season lasts until time $\tau (0 < \tau < 1)$, then it is easily shown that the catch C and abundance at the end of the fishing season N_τ are

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TABLE 1. Effect of adjusting the catch equation for length of the fishing season on calculations of abundance at end of fishing season. The abundance at the end of the year is computed as a proportion of abundance at the beginning of the year for several annual harvest rates. A natural mortality rate of $M = 0.2$ was used.

Catch rate	Length of fishing season (mo)				
	1	3	6	9	12
0.1	0.80	0.78	0.76	0.74	0.73
0.3	0.67	0.64	0.60	0.58	0.55
0.5	0.48	0.46	0.42	0.40	0.37
0.7	0.29	0.27	0.24	0.22	0.20
0.9	0.09	0.08	0.06	0.05	0.04

given by

$$(1) \quad C = N_0 \frac{F}{F+M}(1 - e^{-\tau(F+M)})$$

$$(2) \quad N_\tau = N_0 e^{-\tau(F+M)}$$

where N_0 is the abundance at the beginning of the year and F and M are instantaneous rates of fishing and natural mortality (in units of year^{-1}). The abundance at the end of the year N_1 is simply

$$(3) \quad N_1 = N_0 e^{-(\tau F+M)}.$$

Table 1 illustrates how calculations of abundance depend on the length of the fishing season. For short seasons with moderate catch rates, estimates of abundance at the end of the year, calculated assuming that fishing occurs throughout the year, will be about 90% of the true value. By comparing across rows of Table 1 we can see how much the length of the fishing season affects the total loss from the population. For short seasons with high catch rates, estimated stock size can be as low as 50% of the true value. Adjusting the catch equation for length of season will thus afford an increase in precision for only a slight increase in computational complexity.

Equilibrium Formula for Age-Structured Models

Formulae are here derived for computing values of several model variables at the equilibrium reached when a constant harvest rate is applied indefinitely. Data required include age-specific parameters, such as weight, relative vulnerability to harvesting, and reproductive potential (either egg production or whatever may be chosen to represent "stock" in the stock and recruitment model), and estimates of the stock–recruitment model parameters. Variables to be determined are total biomass before and after fishing, recruitment, total reproductive potential, and the catch. Getz (1980a) provided similar equations except that he assumed that the spawning population was measured after harvest and we assume that spawning occurs before harvest.

The equations depend on the annual order of events. The year starts with recruitment, followed by harvesting. Natural mortality occurs throughout the year. Reproductive potential is assumed to be determined by abundance after recruitment but before the onset of fishing.

Let N_i be the number of fish at age i after recruitment but before harvesting. At equilibrium:

$$(4) \quad N_i = R_\infty, \quad i = r$$
$$= R_\infty \exp\left[-\sum_{j=r}^{i-1}(\tau F V_j + M_j)\right], \quad i = r+1, r+2, \ldots, n$$

where R_∞ = equilibrium recruitment in numbers of fish, V_j = the relative vulnerability to harvesting at age j, M_j = the rate of natural mortality at age j (although M_j will usually be assumed constant over ages), r = the age at recruitment, and n = the oldest age accounted for in the model.

The total equilibrium biomass after recruitment but before fishing is B_∞, given by substituting from 4:

$$(5) \quad B_\infty = \sum_{i=r}^{n} W_i N_i$$
$$= R_\infty Q_1$$

where

$$Q_1 = W_r + \sum_{i=r+1}^{n} W_i \exp\left[-\sum_{j=r}^{i-1}(\tau F V_j + M_j)\right]$$

and W_i = weight at age i.

Total reproductive potential E_∞ is

$$(6) \quad E_\infty = \sum_{i=r}^{n} f_i N_i$$
$$= R_\infty Q_2$$

where

$$Q_2 = f_r + \sum_{i=r+1}^{n} f_i \exp\left[-\sum_{j=r}^{i-1}(\tau F V_j + M_j)\right].$$

If reproductive potential is represented by egg production, then f_i = fecundity at age i adjusted for the sex ratio. If reproductive potential is simply proportional to biomass, then $f_i = W_i$, and if reproductive potential is numbers of fish, then $f_i = 1$.

The biomass of catch is given by

$$(7) \quad C_\infty = \sum_{i=r}^{n} W_i N_i \frac{FV_i}{FV_i + M_i}(1 - \exp[-\tau(FV_i + M_i)])$$
$$= R_\infty Q_3$$

where

$$Q_3 = W_r \frac{FV_r}{FV_r + M_r}(1 - \exp[-\tau(FV_r + M_r)])$$
$$+ \sum_{i=r+1}^{n} W_i \frac{FV_i}{FV_i + M_i}(1 - \exp[-\tau(FV_i + M_i)])$$
$$\times \left(\exp\left[-\sum_{j=r}^{i-1}(\tau F V_j + M_j)\right]\right).$$

Total equilibrium biomass after fishing B_∞' is given by

$$(8) \quad B_\infty' = \sum_{i=r}^{n} W_i N_i'$$
$$= R_\infty Q_4$$

where

$$Q_4 = W_r \exp[-\tau(FV_r + M_r)]$$
$$+ \sum_{i=r+1}^{n} W_i \exp\left[-\tau(FV_i + M_i) - \sum_{j=r}^{i-1}(\tau F V_j + M_j)\right].$$

Note that if breeding takes place after the fishing season, then

N_i' should be used in equation 6 instead of N_i. Substituting f_i for W_i in equation 8 would provide the reproductive potential appropriate for postharvest spawning.

Equations 5–8 have been derived in terms of equilibrium recruitment. It remains to derive an expression for R_∞ in terms of stock and recruitment parameters. If the Ricker (1954) model is used, we have

$$(9) \quad R_\infty = \alpha E_\infty e^{-\beta E_\infty}$$

where α and β are model parameters. Substituting equation 6 into equation 9, we obtain

$$(10) \quad R_\infty = \frac{\ln(\alpha Q_2)}{\beta Q_2}.$$

If the Beverton and Holt (1957) model is used, we have

$$(11) \quad R_\infty = \frac{E_\infty}{(\alpha E_\infty + \beta)}.$$

Substituting equation 6 into 11, we get

$$(12) \quad R_\infty = \frac{Q_2 - \beta}{\alpha Q_2}.$$

Finally, substituting either equation 10 or 12 into equations 5–8, we obtain the equilibrium values in terms of the harvest rate, length of the season, the age-specific parameters, and the stock and recruitment parameters.

Stock–Recruitment Parameters for Age-Structured Models

For age-structured models, the biological interpretation of the parameters α and β is not immediately obvious and can be quite difficult to interpret. Fortunately, α and β can be combined with the other parameters of the age-structured model to calculate more biologically meaningful quantities. There are several possibilities, but we suggest the use of (1) the unfished equilibrium biomass and (2) the rate of biomass recruitment at half the unfished equilibrium biomass. These two quantities, when taken together, are equivalent to α and β, as shown in the next two paragraphs.

The unfished equilibrium biomass can be determined from equation 5 with $F = 0$. The rate of recruitment θ at proportion p of the equilibrium biomass B_∞ (assuming the same age distribution) is given for the Ricker model by equation 13 (details of the derivation are given in the appendix):

$$(13) \quad \theta = W_r \frac{(\alpha Q_2)^{1-p}}{Q_1}.$$

And for the Beverton Holt model:

$$(14) \quad \theta = W_r \frac{Q_2}{Q_1} \frac{1}{(pQ_2 + (1-p)\beta)}.$$

For modeling purposes, it would be convenient if we could set α and β from values of B_∞ and θ. For the Ricker model, it can be shown (details in appendix) that

$$(15) \quad \alpha = \frac{1}{Q_2}\left(\frac{Q_1\theta}{W_r}\right)^{1/(1-p)}$$

and

$$(16) \quad \beta = \frac{Q_1}{Q_2} \ln\left(\frac{Q_1\theta}{W_r}\right) \frac{1}{(1-p)B_\infty}$$

while for the Beverton and Holt model:

$$(17) \quad \alpha = \frac{Q_1}{B_\infty}\left[1 - \frac{1}{1-p}\left(\frac{W_r}{Q_1\theta} - p\right)\right]$$

and

$$(18) \quad \beta = \frac{Q_2}{1-p}\left(\frac{W_r}{Q_1\theta} - p\right).$$

Simplification to Surplus Production Models

It is increasingly recognized that complex age-structured models can be compressed into a much more manageable form with little sacrifice in accuracy. Deriso (1980) presented the most elegant reduced form, compressed a generalized age-structured model into a delay-difference equation with seven parameters. Hilborn (1979), Ludwig and Hilborn (1983), and Ludwig and Walters (1985) have shown that simple two-parameter surplus production models can be used as surrogates for the more complex age-structured models. Hilborn (1979) used a discrete form of the standard Schaefer model:

$$(19) \quad B_{t+1} = B_t + rB_t\left(1 - \frac{B_t}{k}\right) - C_t$$

where B_{t+1} is the biomass at time $t+1$, B_t is the biomass at time t, r is the rate of growth of biomass as B approaches zero, k is the unfished equilibrium stock size, and C_t is the catch at time t.

Ludwig and Hilborn (1983) and Ludwig and Walters (1985) have used Ricker's recruitment model as a surplus production model of the form

$$(20) \quad B_{t+1} = B_t e^{\alpha(1-B_t/\beta)}$$

where e^α is the rate of growth of biomass as B approaches zero and β is the unfished stock size.

There are several ways to compress the behaviour of the age-structured model into the simple surplus production model. We suggest the following.

The properties of the Schaefer model are

$$B_\infty = k$$

$$(21) \quad C^* = \frac{rk}{4}$$

$$B^* = \frac{k}{2}$$

where B_∞ is the unfished stock size, C^* is the maximum sustainable yield, and B^* is the biomass at maximum sustainable yield. Parameter B_∞ can be derived from the age-structured model by setting $F = 0.0$. Parameters C^* and B^* can both be found by a numerical search over values of F. Once B_∞, C^*, and B^* are determined, the values of r and k can be computed using (21).

The parameters of the Ricker equation can be calculated in several ways. The simplest is to use the following equation from Ricker (1975):

$$(22) \quad \alpha = u^* - \ln(1 - u^*)$$

where u^* is the annual harvest rate C^*/B^*. The β of the Ricker model is the unfished stock size and therefore B_∞.

Equilibrium Properties of Pacific Halibut

Age-specific population parameters for the Pacific halibut fishery (R. B. Deriso, International Pacific Halibut Commis-

TABLE 2. Weight, vulnerability, and reproductive potential schedules for Pacific halibut. Reproductive potential is the weight times the percent of females who reproduce at that age. Natural mortality rate (M) was assumed to be 0.2 for all ages.

Age	Average weight (kg)	Relative vulnerability	Reproductive potential
8	8.0	0.18	1.0
9	10.3	0.32	2.6
10	12.7	0.47	4.8
11	15.8	0.59	7.9
12	19.5	0.76	12.2
13	22.7	0.81	17.1
14	26.4	0.92	23.1
15	28.7	1.00	28.7
16	30.8	1.00	30.8
17	33.8	1.00	33.8
18	41.3	1.00	41.3
19	45.7	1.00	45.7
20	44.7	1.00	44.7

sion, University of Washington, Seattle, WA, pers. comm.) are listed in Table 2. Recruitment to the gear occurs at age 8, and the oldest age in the model is 20. Natural mortality is assumed constant over all ages, with $M = 0.2$. The length of the fishing season is currently about 4 wk, so $\tau = 4/52 = 0.0769$.

Reproductive potential for the stock and recruitment model was defined to be the sum over ages 8–20 of a fraction of the total biomass at each age, where the fraction begins at 1/8 for age 8, and increases by 1/8 for each age from age 9 to age 14, and is equal to unity for ages 15–20. Recruitment in thousands of fish is determined from a Ricker model with the parameters $\alpha = 0.187$ and $\beta = 0.016$.

The optimal harvest rate is not available in closed form, so the equilibrium properties were calculated numerically by varying F between 0 and 22 in increments of 0.25. The apparently high values of F are a result of the very intense but short fishing season. The unfished stock size (B_∞) is 226 KMT, and the maximum sustainable harvest (C^*) is 35 KMT, which takes place when $F^* = 8.0$. The biomass at optimum harvest rate (B^*) is 147 KMT, and the annual harvest rate at optimum (u^*) is 0.24.

The alternative stock–recruitment parameters were also calculated. The unfished equilibrium biomass is 226 KMT, and the rate of recruitment at half the unfished equilibrium biomass (θ) is 0.30. The latter value seems reasonable for a long-lived species. For short-lived, highly fecund species, we would expect θ to be larger.

Figure 1 shows the yield-isopleth diagram for the fishery, where the x-axis represents the annual catch divided by the preharvest biomass (harvest rate), and the y-axis represents the first year of vulnerability. The vulnerability of all older ages is unchanged. While this is somewhat unrealistic, it does let us examine the potential increase in yield that could be obtained by increasing the age at first capture. Although this is not a yield-per-recruit diagram, its general shape is quite similar.

Figure 2 shows the equilibrium stock size and the equilibrium yield versus harvest rate. This curve conforms to the general shapes we have come to expect from surplus production models.

Given the relationship between the Schaefer parameters r and k and the three numbers available from the age-structured model (B_∞, B^*, C^*), there are four possible combinations of r and k

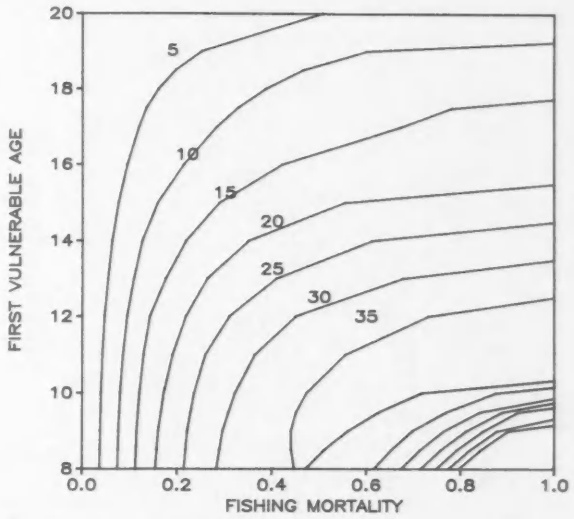

FIG. 1. Yield-isopleth diagram for Pacific halibut using the data from Table 2. Contours are in thousands of tonnes per year. Fishing mortality rate is τF.

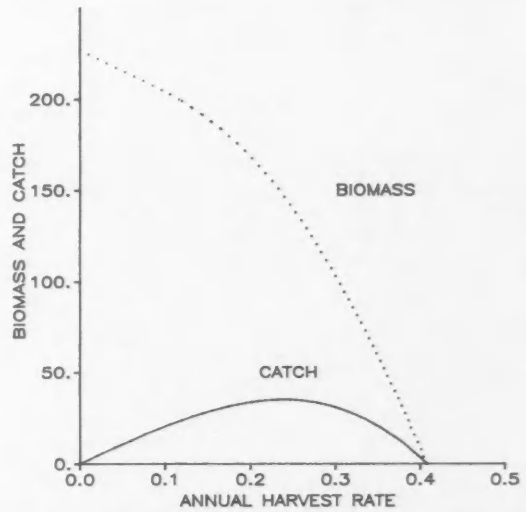

FIG. 2. Equilibrium stock size and yield versus harvest rate for Pacific halibut. Annual harvest rate is the catch divided by the preharvest biomass.

that can be calculated. These are shown in Table 3, along with the parameters that can be calculated from r and k using the Schaefer model. In each set, some of the biologically meaningful parameters (B_∞, B^*, C^*, and u^*) are equal to those of the "real" age-structured model, and some of the parameters are not the same. The best approach is the upper right-hand set, in which k is estimated by multiplying the optimum stock size of the age-structured model by 2, and r is obtained by multiplying the ratio of catch to biomass at optimum by 2.

While there may appear to be some circularity in these calculations, whenever the use of the surplus production model is desired, the best way to calculate parameters may be to use the age-structured model. The surplus production model would be

TABLE 3. Optimal equilibrium properties of Schaefer model parameters using parameters derived from equilibrium properties of an age-structured model. The Schaefer parameters r and k can each be estimated in two different ways from B_∞, C^*, and B^*.

	$k = B_\infty = 227$	$k = 2B^* = 296$
$r = \frac{2C^*}{B^*} = 0.48$	$B_\infty = 226$ $C^* = 27$ $B^* = 113$ $u^* = 0.24$	$B_\infty = 296$ $C^* = 35$ $B^* = 147$ $u^* = 0.24$
$r = \frac{4C^*}{B_\infty} = 0.62$	$B_\infty = 226$ $C^* = 35$ $B^* = 113$ $u^* = 0.31$	$B_\infty = 296$ $C^* = 46$ $B^* = 147$ $u^* = 0.31$

used for optimization, simulation, or gameing. Therefore we want to have our compressed surplus production model behave as much like the full age-structured model as possible. The upper right hand set in Table 3 is probably preferred because we would like our compressed model to have the same optimum stock size, sustainable yield, and optimum harvest rate as the full age-structured model.

Using equation 22 to estimate the parameters of a Ricker-type surplus production model produces $B_\infty = 226$, $C^* = 33$, $B^* = 105$, and $u^* = 0.24$. In this case the optimum stock size and yield are underestimated.

The utility of any of these surplus production representations of the full age-structured model will depend on the exact use of the compressed model, and the choice of which parameter estimation method to use is left to potential users. Potential users are reminded that many other methods can be used, including a Beverton–Holt stock–recruitment curve as a surplus production model, or the Pella and Tomlinson (1969) surplus production model.

Discussion

We have presented a simple method for summarizing age-structured data for harvested populations. Weight and fecundity schedules are usually easily obtained from data collected in association with age sampling. If a time series of age composition of catch is available, the remaining data required can be determined by the method of Fournier and Archibald (1982), or by cohort analysis (Pope 1972) used in conjunction with a method for the estimation of stock–recruitment parameters (Ludwig and Walters 1981).

Model dynamics will in general depend largely on the relationship between the fecundity and vulnerability schedules. Density-dependent growth and species interactions are the two biological factors not included in this analysis that may be significant. Nevertheless, useful statistics characterizing the population are the equilibrium properties and alternative stock–recruitment parameters as described above. The equations are simple enough to solve on a programmable calculator, and yet enable one to analyze yields for different vulnerability schedules and fishing intensities.

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Appendix: Derivation of Stock–Recruit Parameters for Age-Structured Models

For the Ricker stock–recruitment curve (equation 9), the rate of biomass recruitment at proportion p of equilibrium biomass, assuming the same harvest rate F, is given by

$$\theta = \frac{W_r R_\theta}{pB_\infty} \tag{A.1}$$

where R_θ = recruitment in numbers of fish at proportion p of the unfished biomass. It remains to express R_θ in terms of α, β, and p and B_θ in terms of α and β.

To begin, substituting equation 10 into equation 5, we obtain

$$B_\infty = \frac{Q_1}{Q_2}\frac{\ln(\alpha Q_2)}{\beta}. \tag{A.2}$$

Now, rewriting R_θ, we have

$$R_\theta = \alpha E_\theta e^{-\beta E_\theta} \tag{A.3}$$

where E_θ = reproductive potential at proportion p of the equilibrium biomass. Assuming the same age distribution as for B_θ, then

$$E_\theta = \sum_{i=r}^{n} f_i p N_i = pE_\infty. \tag{A.4}$$

Substituting equation 10 into equation 6 and then using the result in equation A.4 gives

$$E_\theta = p\frac{\ln(\alpha Q_2)}{\beta}. \tag{A.5}$$

Now, rewriting equation A.3 using equation A.5, we obtain

$$(A.6) \quad R_\theta = \frac{\alpha}{\beta} p \ln (\alpha Q_2)(\alpha Q_2)^{-p}.$$

Finally, substituting equation A.2 and equation A.6 into our expression for θ, we derive

$$(A.7) \quad \theta = W_r \frac{(\alpha Q_2)^{1-p}}{Q_1}.$$

As derived above, equation A.2 and equation A.7 are valid for any value of the harvest rate. For B_∞ and θ to refer specifically to the *unfished* equilibrium biomass and rate of recruitment at proportion p of the *unfished* equilibrium biomass, respectively, we need only write Q_1 and Q_2 with $F = 0$.

Following the same line of argument, for the Beverton–Holt curve (equation 11), we have

$$(A.8) \quad B_\infty = \frac{Q_1}{Q_2} \frac{(Q_2 - \beta)}{\alpha}$$

and

$$(A.9) \quad R_\theta = \frac{E_\theta}{\alpha E_\theta + \beta} = p \frac{(Q_2 - \beta)}{\alpha} \cdot \frac{1}{pQ_2 + (1-p)\beta}.$$

Finally, substituting equation A.8 and equation A.9 into our expression for θ, we obtain

$$(A.10) \quad \theta = \frac{W_r Q_2}{Q_1} \cdot \frac{1}{pQ_2 + (1-p)\beta}.$$

To derive α and β in terms of θ and B_∞ for the Ricker curve, rewriting equation A.7 gives α directly (equation 15). To derive β, simply solve the equation obtained when equation 15 is substituted into equation A.2.

Similarly, to derive α and β in terms of θ and B_∞ for the Beverton–Holt curve, first solve equation A.10 for β. Then to derive α, solve the equation obtained when equation 18 is substituted into equation A.8.

Fish Hepatocytes: A Model Metabolic System[1]

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The hepatocyte or liver cell preparation is a standard metabolic model in mammalian physiology/biochemistry. This paper presents a basic method for the isolation of viable fish hepatocytes, reviews specifically the literature available on hepatic function and adaptation using the preparation, and examines those areas where this preparation could contribute to our understanding of basic and applied fisheries biology. Viable liver cells are prepared by collagenase perfusion and collected by low-speed centrifugation. Buffered salines employed for cell isolation must be consistent with the normal plasma constituents of the species. Recent studies have established that a variety of fish hepatocytes remain metabolically active in suspension and perform a variety of anabolic tasks, including gluconeogenesis, lipogenesis, ketogenesis, sterol biosynthesis, synthesis of export and mitochondrial proteins, and nucleic acid biosynthesis. New techniques, including cell culture, will provide biologists with a tool to study the more long-term effects of hormones, xenobiotics, and micronutrient requirements under controlled laboratory conditions. The use of fish hepatocytes to reduce the necessity of whole animal models in pharmacological and toxicological studies is particularly promising. The fish hepatocyte preparation represents an important tool to further our understanding of the role of the liver in the biology of fish species.

La préparation d'hépatocytes ou de cellules du foie est un outil métabolique utilisé couramment pour étudier la physiologie et la biochimie des mammifères. Le présent article décrit une méthode de base permettant d'isoler des hépatocytes de poisson viables, passe en revue en particulier les ouvrages publiés sur la fonction et l'adaptation des cellules hépatiques étudiées à l'aide de cette technique de préparation et examine les secteurs où celle-ci pourrait nous permettre de mieux comprendre la biologie fondamentale et appliquée des pêches. On prépare les cellules hépatiques viables en se servant de la technique de perfusion par collagénase et on les recueille par centrifugation à faible vitesse. Les solutions salines tamponnées utilisées pour isoler les cellules doivent être compatibles avec les constituants normaux du plasma des espèces. Des études récentes ont montré que divers hépatocytes de poisson demeurent actifs en suspension sur le plan du métabolisme et accomplissent diverses fonctions anaboliques, comme la glucogenèse, la lipogenèse, la cétogenèse, la biosynthèse de stérols, la synthèse de protéines constituant les mitochondries et de protéines destinées à sortir de la cellule ainsi que la biosynthèse d'acides nucléiques. De nouvelles techniques, y compris la culture de cellules, fourniront aux biologistes un outil leur permettant d'étudier les effets des hormones à plus long terme, la xénobiotique et les besoins en oligoéléments dans des conditions contrôlées en laboratoire. L'utilisation d'hépatocytes de poisson permettant de moins avoir recours à des modèles se servant d'animaux entiers dans les études pharmacologiques et toxicologiques est particulièrement prometteuse. La préparation d'hépatocytes de poisson constitue un outil important qui nous permettra de mieux comprendre le rôle du foie dans la biologie des espèces de poissons.

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Berry and Friend first described the isolation of liver parenchymal cells by collagenase perfusion in 1969. The use of intact hepatocytes has now been established as an ideal system for the study of many aspects of hepatic metabolism. The advantages of using isolated liver cells over other tissue preparations are many (Harris and Cornell 1983) and offset any limitations thus far discovered.

After a lag phase of a number of years, the isolation technique has been successfully adapted to piscine systems (Birnbaum et al. 1976; Hayashi and Ooshiro 1978; Bouche et al. 1979; Walton and Cowey 1979a; Campbell et al. 1983) and it is just now that fish researchers have begun to employ hepatocytes, either freshly isolated or in primary culture, to their full potential. The widespread interest is reflected in the recent flourish of publications employing this powerful, yet simple, experimental tool.

The purpose of this review is to characterize the fish hepatocyte preparation, to indicate its general use in the study of hepatic function in fish, and to provide an insight into future areas of research where it may be employed. The general treatment of the literature should provide the background necessary for significant future contributions to this field by fishery scientists.

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TABLE 1. Perfusion media for the isolation of viable salmonid hepatocytes. Modified from French et al. (1981).

Medium A (perfusion, first separation)
Modified Hanks medium (balanced salts) (Hanks and Wallace 1949)
176 mmol $NaCl \cdot L^{-1}$
5.4 mmol $KCl \cdot L^{-1}$
0.81 mmol $MgSO_4 \cdot L^{-1}$
0.44 mmol $KH_2PO_4 \cdot L^{-1}$
0.35 mmol $Na_2HPO_4 \cdot L^{-1}$
4–8 mmol $NaHCO_3 \cdot L^{-1}$, depending upon fish species*
10 mmol $HEPES \cdot L^{-1}$ added to Hanks medium
*$NaHCO_3$ is added after gassing the medium with 0.5–1% CO_2 (balance O_2) for 30 min; pH is adjusted to pH 7.6 at 20°C
Medium B (washing and incubation)
Medium A to which is added
2% bovine serum albumin, fatty acid free
1 mmol $CaCl_2 \cdot L^{-1}$
Medium C (digestion)
Medium A to which is added
0.6 mg collagenase $\cdot mL^{-1}$ (*Clostridium* sp.)

Preparation of Viable Fish Liver Cells

Although isolation procedures for the preparation of fish hepatocytes have been previously described (Birnbaum et al. 1976; Hayashi and Ooshiro 1978; Walton and Cowey 1979a; Renaud and Moon 1980a; French et al. 1981), we feel it is necessary to review the general technique, to include a few words of caution, and to achieve some unifying concept in the approach.

Choice of Perfusion Medium

The most appropriate perfusion media are those that mimic most closely the plasma composition of the respective fish. Balanced salt solutions, as outlined by Lockwood (1961), Wolf (1963), King et al. (1980), and others, constitute the basic solution. These solutions are buffered, with HEPES for example, and adjusted to a hydrogen ion concentration that approaches those in vivo. This results in substantially higher pH values than commonly employed for mammalian studies. Also, the thermal history of the fish species under consideration must be taken into consideration. Table 1 presents the buffered salt solutions used in our laboratories for the isolation of viable salmonid hepatocytes (French et al. 1981). Slight modifications of these allow their general use. Often neglected is the fact that fish blood is characterized by low bicarbonate concentrations and concomitant low pCO_2 values (Albers 1970). Therefore, low concentrations of bicarbonate (4–8 $mmol \cdot L^{-1}$) and CO_2-poor gas phases (0.5–1% CO_2, remainder O_2) should be chosen, both of which approach physiological conditions (Table 1). Major differences in metabolite flux are reported at high and low bicarbonate concentrations (Mommsen and Suarez 1984). While the importance of exogenous glucose to liver metabolism is under debate, it may be advantageous to include a carbon source (glucose, lactate, or amino acids) in the perfusion medium.

Calcium ions in the perfusion medium may jeopardize the isolation of cells by two different modes of action, and consequently are generally not included in the preparation medium. First, cells are less likely to disaggregate sufficiently after perfusion in the presence of calcium, leading to low yields and clumping, rendering the cells unsuitable for metabolic studies (Seglen 1976). Second, calcium is a strong activator of microbial collagenase, which complicates the isolation procedure by requiring the removal of the collagenase and/or calcium prior to the purification of the hepatocytes by differential centrifugation.

Procedure

After the fish is anesthetized, a ventral incision is made and the intestinal vein, the hepatic portal vein, or another suitable vessel is cannulated. Subsequently, the liver is cleared of blood by perfusion with well-oxygenated medium A (Table 1). With experience, the heparinization of the experimental animal before cannulation or the inclusion of heparin in the perfusion medium will become unnecessary and the side effects introduced by heparin can be avoided. The flow rate of the perfusate is adjusted to guarantee fully aerobic conditions for the liver cells during the course of the isolation (2 $mL \cdot min^{-1} \cdot g\ liver^{-1}$). At this point, gentle massage of the liver will strongly enhance blood clearance, decrease red blood cell contamination of the isolated cells, and also substantially increase the yield.

After all the blood has been cleared, the perfusion medium is switched to include collagenase (medium C, Table 1). The concentration of collagenase is chosen (usually less than 0.6 $mg \cdot mL^{-1}$) to deliver a well-digested liver within 30–45 min of perfusion. At this point, vessel stability and proximity of the cannula to the liver permitting, the liver can be removed from the animal and placed onto a watchglass. The perfusion with medium C is continued, if necessary under recirculation, until the liver shows the first signs of disintegration. The liver is placed into a petri dish, doused with ice-cold medium A, and after careful removal of the gall bladder, minced with a razor blade. Poorly perfused areas are removed and the remainder is poured through two successive screens of plankton netting (253 and 73 μm, respectively). Yields are increased by gently massaging the pieces of tissue through the coarser screen. Cells are collected by centrifugation (40–100 × *g*, depending on the species) for 2 min at 0–4°C. The resulting supernatant is carefully decanted and the cells gently resuspended in medium B (Table 1). The hepatocytes are washed three times with medium B and each time collected by low-speed centrifugation. Gradient centrifugation is another route leading to cleaned hepatocytes (Bouche et al. 1979). If during any of these steps clumping of the cells occurs, the preparation should be abandoned.

Finally, the cells are suspended in a small volume of medium B, weighed, counted on a hemocytometer, and tested for viability (Table 2). If analysis of metabolic performance is to be assessed, the cell concentration is adjusted to 25–50 mg fresh weight $\cdot$ mL suspension $medium^{-1}$.

Criterion for Viability

The viability of freshly isolated hepatocytes and their suitability for metabolic studies is commonly assessed by testing a number of different criteria (Table 2). However, most of these fail to convey information about cell integrity on different organizational levels (e.g. the intactness of hormone receptors). In addition, freshly isolated rat hepatocytes tend to be in negative nitrogen balance, the cells may lose intracellular potassium during the isolation procedure, and membrane potential may be adversely affected (Schwarze and Seglen 1980; Cornell 1983). Unfortunately, the analyses done on shortcomings of the isolated cells have emphasized rat or other mammalian species, and few fish studies have adequately surveyed all or most criteria (see Campbell et al. 1983).

TABLE 2. Criteria used to establish hepatocyte viability.

1. Dye exclusion (trypan blue)
2. Exclusion of succinate
3. Low rates of leakage of cytosolic and mitochondrial enzymes into the incubation medium
4. Performance of metabolic processes which require functionality of different cellular compartments (e.g. gluconeogenesis)
5. ATP concentrations not significantly different from the freeze-clamped liver
6. Maintenance of intracellular hydrogen ion concentration (pHi)
7. Maintenance of intracellular potassium ion concentration
8. Polysomal aggregation profiles

Cornell (1983) proposed that the maintenance of intracellular ATP levels is the most sensitive criteria of viability. Figure 1 indicates that American eel (*Anguilla rostrata*) hepatocyte ATP content increases slightly during incubation as does energy charge due to slight decreases in both ADP and AMP. Adenylates have been used as a criteria in many fish cell studies and should become the method of choice (Birnbaum et al. 1976; Renaud and Moon 1980a; van Waarde and Kesbeke 1981; Campbell et al. 1983).

Fish Liver Metabolism

Just as their mammalian counterparts (Harris and Cornell 1983), fish hepatocytes remain metabolically active in suspension for at least a number of hours and perform a wide variety of anabolic functions. In this section we consider the metabolic pathways that have been identified to date. The sensitivity of the hepatocyte system to hormones and the ease of manipulating the extracellular milieu allow this preparation to delineate metabolic differences between species and groups. In the following section, therefore, we examine more closely the regulations of these pathways and the distinction between species.

Gluconeogenesis/Glycolysis

Fish species utilize protein and lipid as energy sources, but poorly utilize carbohydrates (Cowey and Sargent 1979; Walton and Cowey 1982). White muscle, the major tissue mass of fish, is poorly perfused and anerobic, and relies on glycogen breakdown to maintain contractile activities. The ultimate source of muscle glycogen is glucose produced by gluconeogenesis from precursors including amino acids (exogenous and endogenous) and lactate (from anerobic glycolysis). Liver, and possibly kidney, are the sites of gluconeogenesis in vertebrates, including fish.

The estimation of gluconeogenic flux in fish hepatocytes is complicated by high liver glycogen content and the continuous depletion of glycogen during incubation (Birnbaum et al. 1976; Renaud and Moon 1980a; French et al. 1981; Ottolenghi et al. 1984; T. P. Mommsen, unpubl. data). Glycogenolysis can be modified by hormones and calcium (e.g. Birnbaum et al. 1976), substrate availability (e.g. Renaud and Moon 1980a), and pH (Seglen 1972), but fasting cannot deplete reserves as in mammalian studies. The high rates of glucose production from glycogen require radioisotope use to estimate gluconeogenic flux in fish, and complicate studies of glucose utilization (T. P. Mommsen, unpubl. data).

Gluconeogenic flux has been estimated from a number of radioactive precursors in a number of fish species (Table 3). Rates are species dependent, and can be altered by fasting (Renaud and Moon 1980b) and anorexia (French et al. 1983).

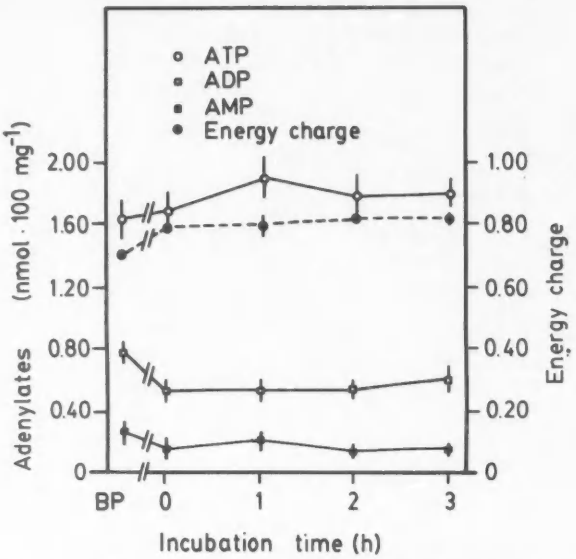

FIG. 1. Effects of incubation time on American eel hepatocyte adenylate contents. BP = before perfusion content. Mean ± SE of four separate experiments. Modified from Renaud and Moon (1980a).

Generally, flux from lactate exceeds that from any amino acid for a particular species (Table 3). As with mammals (e.g. Elliott et al. 1976), alanine and serine are generally the preferred amino acid substrates, possibly due to the ease of transformation into pyruvate or its equivalent (Walton and Cowey 1979b).

Inhibitor studies indicate that amino acid entry into the gluconeogenic pathway is through the respective aminotransferases (Hayashi and Ooshiro 1979; Walton and Cowey 1979a, 1979b; Renaud and Moon 1980a; Hayashi et al. 1982; Mommsen and Suarez 1984). Walton and Cowey (1979b) used enzyme compartmentation and inhibitors to support their view that serine conversion to glucose is initiated by the serine–pyruvate transaminase pathway rather than serine dehydratase as has been reported in mammalian liver.

The relatively low gluconeogenic flux from amino acids compared with lactate may relate to the compartmentation of key gluconeogenic enzymes and cystolic NADH (Mommsen and Suarez 1984). The key gluconeogenic enzyme, phosphoenol pyruvate carboxykinase (PEPCK), is localized to either cystolic or mitochondrial compartments in vertebrate livers (Söling and Kleineke 1976). Where PEPCK has been studied in fish, it is primarily mitochondrial (Phillips and Hird 1977; Walton and Cowey 1979a; Moon and Johnston 1980; Moon 1983; Mommsen and Suarez 1984), with the exception of the Japanese eel, *Anguilla japonica*, where activities are nearly equally distributed in both compartments (Hayashi and Ooshiro 1979). Quinolinate, an inhibitor of cytosolic PEPCK but not the mitochondrial enzyme, inhibits gluconeogenic flux in eels (Hayashi and Ooshiro 1979; Renaud and Moon 1980a), but not in rainbow trout, *Salmo gairdneri* (Mommsen and Suarez 1984). This observation supports the distinct localization of this enzyme in these two species. Similar species differences are observed in mammals and birds (Söling and Kleineke 1976). These studies imply that the fish and mammalian gluconeogenic pathways are qualitatively similar, although quantitative differences obviously exist (Table 3).

TABLE 3. Flux of labelled carbons into glucose, CO_2, and other products by fish hepatocytes. Units are micromoles product per gram wet weight per hour of incubation (generally at 10°C) (L = lipids).

Species	Glucose	CO_2	Other	References[a]
Rainbow trout (*Salmo gairdneri*)				
Lactate	1.2–20.3	11.1, 25.1	—	2a, 4, 6
Pyruvate	10.3	—	—	2a
Alanine	0.2–8.8	2.2–5.8	—	2a, 4, 6
Serine	1.0–5.7	8.5	—	2b, 4
Others				4
Sockeye salmon (*Oncorhynchus nerka*)				
Lactate	0.9–16.5	4.4–12.0	—	10, 5
Alanine	0.4–4.7	0.2–5.7	—	10, 5
Serine	0.5–1.5	1.4–2.8	—	5
Palmitate	—	0.3–1.4	—	5
Coho salmon (*Oncorhynchus kisutch*)				
Lactate	7.7	27.6	—	10
Alanine	1.2	7.6	—	10
Atlantic salmon (*Salmo salar*)				
Lactate	1.1	3.8	—	7
Alanine	0.04	0.8	—	7
Sea raven (*Hemitripterus americanus*)				
Glucose (C-1)	—	0.04	—	8
Lactate	0.12	0.8	—	8
Alanine	0.11	0.7	—	8
Serine	0.60	3.7	—	9
Oleate	—	0.005	—	8
Japanese eel (*Anguilla japonica*)				
Lactate	23.8	—	—	1
Pyruvate	15.9	—	—	1
Alanine	11.4	—	—	1
American eel (*Anguilla rostrata*)				
Lactate	0.6–13.0	0.3–1.9	5.9 (L)	3
Glycerol	19.4	1.1	4.7 (L)	3
Alanine	0.1–12.4	0.05–6.9	7.4 (L)	3
Aspartate	0.07–1.1	nil–0.9	4.5 (L)	3
Rat (*Rattus rattus*) (25°C)				
Lactate	107	—	—	11
Glycerol	78	—	—	11
Alanine	64	—	—	11
Serine	39	—	—	11

[a]1, Hayashi and Ooshiro (1979); 2, Walton and Cowey (1979a, 1979b); 3, Renaud and Moon (1980a, 1980b); 4, French et al. (1981); 5, French et al. (1983); 6, Mommsen and Suarez (1984); 7, Mommsen et al. (1985); 8, Walsh et al. (1985); 9, G. Foster and T. W. Moon (unpubl. data); 10, T. P. Mommsen (unpubl. data); 11, Elliott et al. (1976).

Mommsen and Suarez (1984) have argued that the main role of gluconeogenesis in the rainbow trout is to convert lactate, a product of active muscle contraction, back to glucose. A recent study of glucose and lactate turnover in the American eel found that even though 35% of the lactate utilized in vivo was converted to glucose, this represented less than 1% of the total glucose produced; amino acids were thought to contribute the majority of carbon to glucose (Cornish and Moon 1985). Similar studies need to be undertaken with trout to establish the extent of recycling and validate the hypothesis of Mommsen and Suarez (1984). Obvious locomotor differences exist between salmonids and eels, as well as differences in conversion rates of lactate and amino acids to glucose (Table 3), suggesting distinct gluconeogenic roles for the livers of these species. It should be

remembered that only amino acids can provide de novo synthesis of glucose and glycogen, not lactate which is derived from these compounds.

Another species difference observed in Table 3 is the rate of substrate oxidation. Unlike mammals where there is major label dilution at the oxaloacetate branchpoint (Phillips and Hird 1977; Hetenyi et al. 1983), this apparently is not a difficulty with those fish preparations studied to date. The rate of $^{14}CO_2$ production from a labelled substrate is equivalent to its oxidation (Phillips and Hird 1977; French et al. 1983; Mommsen et al. 1985; T. P. Mommsen, unpubl. data). In most studies to date, the rate of substrate oxidation exceeds gluconeogenesis. However, as flux rates are presented as micromoles glucose or CO_2 per hour per gram wet cell weight, there are five to six times more substrate molecules going to glucose than CO_2 in the salmonid studies (French et al. 1983). The American eel is a major exception to this trend, and ratios of gluconeogenic flux to oxidation generally exceed 1.0 for both lactate and alanine (Renaud and Moon 1980a, 1980b). Renaud and Moon (1980b) reported that the correlation between substrate oxidation and gluconeogenesis approaches the theoretical values needed to balance catabolic and anabolic processes. This has not been established for any other species, although fat oxidation is thought to promote gluconeogenesis in other species (Williamson 1967; Mommsen and Suarez 1984). As the eel liver does not apparently have a complete Krebs cycle (Moon and Ouellet 1979), unlike the trout (Suarez and Hochachka 1981), this may contribute to the differences reported in the catabolic–anabolic ratios in the hepatocytes of these species.

Conversion of precursors to glycogen has been observed in fish hepatocytes. Bouche et al. (1979) reported that ^{3}H-2,3-aspartic acid was incorporated into glycogen of isolated carp (*Cyprinus carpio*) hepatocytes, but whether this was quantitatively significant cannot be assessed from this data. Similarly, isolated catfish (*Ictalurus melas*) (Ottolenghi et al. 1984), eel (Renaud and Moon 1980a), and coho salmon (*Oncorhynchus kisutch*) (Plisetskaya et al. 1984) hepatocytes produce small quantities of glycogen which is dependent upon extracellular glucose concentrations and/or insulin. Glycogen depletion, high medium glucose, and the presence of insulin are all necessary for rat hepatocyte production of glycogen (e.g. Johnson et al. 1972). The negative glycogen balance of fish hepatocytes previously discussed precludes an accurate assessment of glycogen production, even in these studies.

These studies provide evidence that the fish hepatocyte is a tool to study gluconeogenesis. High and continuous glycogen depletion make studies of glycolysis suspect. Lactate is the preferred gluconeogenic substrate, just as in mammals (Table 3), although amino acids including alanine and serine contribute to glucose production in a major way in many species. In fact, gluconeogenesis from serine is higher in those fish species where examined than in mammals when temperature differences are considered. The pathway of gluconeogenesis is species dependent, but patterns are consistent with those observed in bird and mammal studies (Söling and Kleineke 1976; Harris and Cornell 1983).

Protein and Amino Acid Metabolism

A number of fish hepatocyte preparations have demonstrated amino acid incorporation into cell protein. Incorporation of radiolabelled amino acids into the TCA-insoluble fraction has been shown by hepatocytes from carp (Bouche et al. 1979; Saez et al. 1982), European eel (*Anguilla anguilla*) (Jankowski et al. 1981), catfish (Koban and Prosser 1983), and juvenile coho salmon (Plisetskaya et al. 1984; Bhattacharya et al. 1985). Haschemeyer and Mathews (1983) found that ^{14}C-leucine incorporation into hepatocyte protein of two Antarctic fishes (*Trematomus hansoni* and *T. bernacchii*) proceeded at a rate about 15% of the observed in vivo rate. After culturing the cells on modified medium, rates were increased to as much as 50% of the in vivo rate. Gravid fish incorporated radioactive amino acids into vitellogenin for export purposes. T. P. Mommsen and C. B. Lazier (unpubl. data) have also shown vitellogenin production by Atlantic salmon (*Salmo salar*) hepatocytes, and that production was stimulated by estradiol.

Other studies have shown specific protein synthesis in fish hepatocyte preparations. These include antifreeze glycoproteins (O'Grady et al. 1982), heat-shock proteins (Koban et al. 1984), and mitochondrial proteins (Kent and Prosser 1980).

Amino acid transport has received some attention. Haschemeyer and Detrich (1982) examined ^{14}C-leucine uptake by Antarctic fish hepatocytes. The transport system was saturable, temperature sensitive, and subject to competition by other amino acids. Similar studies have been reported for β-alanine transport into isolated hepatocytes of skates (*Raja erinacea*) (Shuttleworth and Goldstein 1984) and alanine/α-aminoisobutyric acid into eel hepatocytes (T. W. Moon, unpubl. data). Plisetskaya et al. (1984) demonstrated insulin sensitivity of ^{14}C-cycloleucine transport, a nonmetabolizable amino aid analogue, in juvenile coho salmon.

Ammonia production from amino acids has been studied with catfish (Campbell et al. 1983; Casey et al. 1983) and goldfish (*Carassius auratus*) (van Waarde and Kesbeke 1981) hepatocytes. The use of inhibitors demonstrated in both species that the purine nucleotide cycle played a minor role compared with transdeamination pathways in ammonia production.

It is a paradox that even though protein and amino acids play pivotal roles in the metabolism of fish species (e.g. Walton and Cowey 1982), few critical questions have been addressed using the hepatocyte preparation. Interest has been placed on their metabolism (see Gluconeogenesis/Glycolysis), even though there is a large body of evidence supporting transport as the limiting factor to amino acid metabolism (e.g. Kilberg 1982). The studies mentioned provide evidence for the viability of the fish hepatocyte preparation as a tool to study protein and amino acid metabolism. Transport is a major, underdeveloped topic, as is the effects of hormones. Both areas represent important future study directions.

Lipid Metabolism

The oxidation of radioactive fatty acids (lipolysis) has been demonstrated in isolated hepatocytes of the killifish, *Fundulus heteroclitus* (Moerland and Sidell 1981), sockeye salmon, *Oncorhynchus nerka* (French et al. 1983), and the sea raven, *Hemitripterus americanus* (Walsh et al. 1985). Rates are generally less than that for either lactate or specific amino acids (Table 3) and temperature dependent (Moerland and Sidell 1981).

Lipogenesis has been estimated in rainbow trout (Hazel and Prosser 1979; Hazel and Sellner 1979) and American eels (Renaud and Moon 1980a). Carbons from acetate (Hazel and Prosser 1979), lactate, alanine, and aspartate (Renaud and Moon 1980a) are readily incorporated into a variety of lipid products. Hazel and Sellner (1979) employed tritium incorpora-

tion from tritiated water to show that total lipogenesis was temperature dependent, as was the ratio incorporated into fatty acids and sterols. Specific phospholipid synthesis (Hazel 1982) and specific desaturation and elongation pathways (Sellner and Hazel 1982) have been examined in trout hepatocytes.

Jankowsky et al. (1984) found that acetoacetate production was stimulated by oleate addition to isolated European eel hepatocytes. Endogenous ketogenic rates were temperature and acclimation dependent, and paralleled rates of hepatocyte lipolysis. As elasmobranchs and teleosts are quite distinct with regard to ketone body production (Zammit and Newsholme 1979), and little is known of ketone production or function in teleosts, the hepatocyte preparation may represent an important tool in investigating these species differences.

Just as in the case of amino acids, lipid metabolism has received little attention, except for temperature studies (see Temperature Adaptation). Walton and Cowey (1982), in their review of lipogenesis, suggested that fish have a "moderate capacity for lipogenesis." This hypothesis is based upon the abundance of lipid reserves, the close correlation between endogenous lipid content and dietary lipid intake, especially in marine species, and the low carbohydrate intake of fish which is thought (assumed?) to represent a major precursor of lipids. Studies by Aster (1981) with American eels support this hypothesis, even though a number of precursors are actively converted to lipids in liver slice preparations. The lack of information on specific lipid precursors needs to be corrected, and the hepatocyte preparation could provide this important background information.

Regulation of Fish Liver Metabolism

Hepatocytes prepared from either mammals or fish are viable preparations for the study of metabolism. The external medium of hepatocytes can be altered with ease and independent of the myriad of other complicating factors. For these reasons, hepatocytes have become the system of choice to study effectors that may regulate liver metabolism. Extensive work has been reported for mammalian cells (see Harris and Cornell 1983), and recent fish studies are now beginning to investigate interesting species metabolic differences. This section deals specifically with hormones and temperature and their impact upon fish hepatocyte metabolism.

Hormone Regulation

The regulation of liver metabolism is well established in mammals (e.g. Hetenyi et al. 1983; Kraus-Friedmann 1984). Fish responds less consistently to hormones, but the general pattern that is emerging suggests that few differences may exist between the responses of fish and mammals to specific hormones.

The depletion of stored glycogen is the most consistent response noted by fish hepatocytes incubated with epinephrine, glucagon, or c-AMP (as dibutyryl c-AMP). This has been reported for goldfish (Birnbaum et al. 1976), Japanese eel (Hayashi and Ooshiro 1975; Hayashi et al. 1982), and American eel (Renaud and Moon 1980a, 1980b) hepatocytes. The response to epinephrine and c-AMP occurs without a lag phase and at low, presumably physiological concentrations (micromoles per litre and less). Birnbaum et al. (1976) were able to demonstrate that goldfish hepatic glycogenolysis was a β-adrenergic response, just as in mammals.

These same hormones increase glucose release from hepatocytes beyond that derived from glycogen. Lactate and amino acid gluconeogenesis are activated in both rainbow trout (Walton and Cowey 1979a; Mommsen and Suarez 1984) and American eel (Renaud and Moon 1980a, 1980b) hepatocytes, and Morata et al. (1982) found that a pyruvate transport inhibitor, α-cyanocinnamate, blocked the enhanced glucose release due to hormone addition in trout liver slices without changes in glycogen content. Table 4 presents the extent of lactate and alanine gluconeogenic activation of trout hepatocytes, and suggests that both glucagon and c-AMP may also affect the oxidation of these compounds (Mommsen and Suarez 1984). These studies support a strong effect of at least glucagon and c-AMP on fish hepatocyte gluconeogenesis. Mommsen and Suarez (1984) further reported that both glucagon and c-AMP altered activities of pyruvate kinase at low, but not high, substrate concentrations. This observation implies that phosphodephospho regulation of trout liver pyruvate kinase may be an important gluconeogenic control mechanism, just as in mammals (Kraus-Friedmann 1984).

TABLE 4. Effects of glucagon (10 mmol·L^{-1}) and c-AMP (100 mmol·L^{-1}) on gluconeogenesis and CO_2 production from lactate (10 mmol·L^{-1}) and alanine (2 mmol·L^{-1}) in rainbow trout hepatocytes, at 15°C. Modified after Mommsen and Suarez (1984).

Addition	CO_2 (μmol CO_2 released·g^{-1}·h^{-1}) ± SD or range (*n*)	Glucose (μmol formed·g^{-1}·h^{-1}) ± SD or range (*n*)
Lactate		
None	16.77±2.67 (7)	2.92±0.94 (7)
Glucagon	21.30±4.80 (4)[a]	3.79±0.85 (4)[a]
c-AMP	16.77±0.75 (2)	4.15±0.46 (4)[a]
Alanine		
None	3.30±1.38 (4)	0.18±0.16 (4)
Glucagon	8.48 (6.37–10.63) (2)	0.70 (0.46–0.94) (2)
c-AMP	4.39 (4.16–4.69) (2)	0.36 (2)

[a]Significantly different from control value by a paired *t*-test at $P < 0.005$.

Glucagon has also been shown to increase uptake of ^{14}C-α-aminoisobutyric acid (AIB), but not ^{14}C-glycine, into the TCA-soluble (nonprotein) fraction of Japanese eel liver slices (Inui and Ishioka 1983a, 1983b). Inui and Yokote (1977) previously reported that glucagon decreased plasma amino nitrogen in eels, confirming a possible site for glucagon action in fish at the level of membrane transport. Mammalian liver cells transport glycine and AIB possibly on separate carriers, with only the AIB carrier sensitive to glucagon (Kilberg 1982).

The roles of glucagon, c-AMP, and epinephrine are reasonably well studied in fish systems, and the mechanisms by which they alter liver metabolism are apparently similar to those seen in mammals. Further studies are needed to evaluate other enzyme steps within this critical pathway to ensure consistency with the mammalian studies (Kraus-Friedmann 1984). Also, glucagon studies are needed, especially with regard to its phylogenetic appearance within the teleosts, and its possible interrelationships with other hormones.

The role of insulin is more controversial than any of the above hormones. Amino acid transport and protein synthesis are enhanced by insulin in Japanese eel liver slices (Inui and Ishioka 1983a, 1983b) and coho salmon hepatocytes (Plisetskaya et al. 1984). ^{14}C-Cycloleucine uptake was also activated by insulin in both studies. Insulin does activate specific amino acid uptake

into mammalian liver cells (Kilberg 1982), and these studies seem to support a similar mode of action in fish. However, the incorporation of ^{14}C-glycine (Inui and Ishioka 1983b) and ^{14}C-leucine (Plisetskaya et al. 1984) was enhanced only into the TCA-insoluble (protein) fraction, not the TCA-soluble (nonprotein) fraction, suggesting that the primary mode of action of insulin is on protein synthesis (at the expense of intracellular amino acids) and possibly secretion (Plisetskaya et al. 1984), not transport. This difference between fish and mammal liver metabolism needs to be clarified, as do the precise effects of interspecific insulins on the response (Plisetskaya et al. 1984).

The effects of insulin on liver glycogen encompass all possibilities, from no effect to either an absolute increase or decrease. Plisetskaya et al. (1984) found enhanced glycogen synthesis at 10^{-9} mol insulin·L^{-1}, but not 10^{-8} mol·L^{-1}. Ottolenghi et al. (1984) found a decrease in glycogen by insulin addition in catfish hepatocytes. Interestingly, Ottolenghi et al. (1985) followed up these hepatocyte studies with similar but longer term studies using a perfused catfish liver preparation. They found glycogen synthesis with insulin, but only after a 4-h perfusion. What may be happening in these and other experiments is that the insulin receptors become insensitive to insulin during isolation, and require a time period to regain their sensitivity. Plisetskaya et al. (1984) routinely used an initial 3-h period for "metabolic recovery" of liver cells from salmon, which may account for the positive response noted in these but not other experiments. Isolation artifacts have also been reported for mammalian cells which can be reduced by culturing (Ichihara et al. 1982).

Insulin has also been shown to decrease medium glucose in incubating fish hepatocytes. This has been demonstrated in Japanese eels (Hayashi and Ooshiro 1975; Hayashi et al. 1982), American eels (Renaud and Moon 1980a) and catfish (Ottolenghi et al. 1985). The quantitative significance of glucose uptake into the liver is not clear, although some authors (e.g. Ablett et al. 1983) suggest that glucose is directed towards anabolic products other than glycogen. The negative glycogen balance of fish hepatocytes makes the interpretation and design of experiments difficult.

A hypoglycemic role for insulin in fish appears to be supported by these data. The cellular mechanism(s) by which this occurs is not known, nor have species differences been investigated, two areas where the use of the hepatocyte could be of great value.

Few other hormones have been investigated using fish hepatocyte preparations. The uptake of ^{3}H-cortisol was found to involve both low affinity, saturable (K_m = 60 μmol·L^{-1}), and diffusion components in trout hepatocytes (Porthe-Nibelle and Lahlou 1981). Dexamethasone transport was even more rapid and complexly affected cortisol uptake. Considering the importance of the direct and permissive actions of glucocorticoids on mammalian metabolism (Kraus-Friedmann 1984), and their known effects in teleosts (Murat et al. 1981), more studies on these steroid hormones are needed using the fish hepatocyte system.

Bhattacharya et al. (1985) have examined the effects of estradiol (E_2) and triiodothyronine (T_3) on protein synthesis in juvenile coho salmon hepatocytes. Both E_2 and T_3 stimulated radioisotope incorporation from amino acids into TCA-insoluble (protein) materials, but only E_2 stimulated protein export. E_2 has also been found to enhance vitellogenin release from Atlantic salmon hepatocytes (T. P. Mommsen and C. B. Lazier, unpubl. data).

Studies with hormones and fish hepatocytes can answer many fundamental and practical questions in fish metabolism. Sensitivity may be reduced after isolation, or at least modified, resulting in preparations that respond inappropriately to hormone addition. A short "metabolic recovery" may be adequate to overcome this difficulty, or as with mammalian studies (Ichihara et al. 1982), more extensive tissue culture techniques may be required.

Temperature Adaptation

Environmental temperature affects a variety of physiological and biochemical processes that are important from the perspective of fisheries biology. Growth and developmental rates, and the impact of environmental insults (be they biotic, abiotic, or xenobiotic), are markedly temperature dependent. Generally, fish are more susceptible to these effects at higher temperatures (e.g. Hazel and Prosser 1974). As the liver plays a major role in these processes, in this section we examine some of the effects of temperature on fish liver function. A great deal of information concerning temperature adaptation in general is available (Hazel and Prosser 1974; White and Somero 1982; Hochachka and Somero 1984), so we concentrate primarily on the more recent experiments with hepatocytes.

Thermal acclimation results in a wide variety of metabolic changes in the liver. For example, cold adaptation is accompanied by a metabolic reorganization which includes a decrease in oxygen consumption rates (Moerland and Sidell 1981), marked hypertrophy of the liver (Kent et al. 1983; Walsh et al. 1983), a shift from lipid to carbohydrate as a fuel source (Stone and Sidell 1981), enhanced biosynthesis (Hazel and Sellner 1979; Stone and Sidell 1981; Haschemeyer and Mathews 1983; Walsh et al. 1985), homeoviscous adaptation in membrane lipids (Hazel 1984), and a decrease in some enzymic activities associated with detoxification pathways (Hazel and Prosser 1974). Several potential mechanisms may be responsible for the observed changes in the rates of these metabolic processes. Concentrations (and hence activities) of existing enzymes may be varied (quantitative strategy), synthesis of new forms of proteins may be induced (qualitative strategy), or the microenvironment (cytosolic pH, membrane lipid fluidity) of existing enzymes may be modified (modulation strategy) (Hochachka and Somero 1984). Hepatocyte systems have been useful in demonstrating certain mechanistic aspects of these strategies.

Quantitative strategy

The activities of enzymes in tissue homogenates vary with acclimation temperature (Hazel and Prosser 1974), and actual changes in the concentration of specific enzymes have been measured (Wilson 1973; Sidell 1977). Recent studies with hepatocytes suggest that the control of this concentration change rests with the cell itself, rather than with systemic control. Cold acclimation leads to increased glucose-6-phosphate dehydrogenase (G6PD) activity in catfish liver, and this activity increase is attributed to a specific increase in G6PD concentration (Kent et al. 1983; Kent and Prosser 1984). When hepatocytes isolated from catfish acclimated to 15°C are cultured at 7, 15, and 25°C for 20 d, the coldest cells again displayed the highest titres of G6PD (Koban and Prosser 1983).

Qualitative strategy

Induction of new isozymic forms of most metabolic enzymes does not appear to be a widespread strategy employed by fish during thermal acclimation. A notable exception are the tetra-

ploid salmonids (see Hochachka and Somero 1984). However, de novo induction of specialized proteins that protect cellular function may be an important mechanism for tolerance of extremes in temperature. Many aspects of antifreeze proteins have been studied in fish (DeVries 1983), and hepatocytes have recently been used to characterize antifreeze glycoprotein synthesis (O'Grady et al. 1982).

Following heat shock, many cell types produce specific proteins of a class called heat shock proteins (hsp), the function of which is believed to be protective, but as yet is largely unknown (Tanguay 1983). Recently, fish hepatocytes were used to demonstrate that the threshold temperature for hsp production (i.e. the minimum temperature at which synthesis begins) may be modified by the thermal history of the fish. Hepatocytes from catfish acclimated to 7°C produced hsp at 15°C, whereas hepatocytes isolated from catfish acclimated to 25°C showed no hsp synthesis until 35°C (Koban et al. 1984). Furthermore, the electrophoretic pattern of hsp production appeared to be temperature dependent (Koban et al. 1984).

Modulation strategy

In lieu of changes in the concentrations or types of proteins per se, changes in the microenvironment surrounding the proteins have several advantages during thermal acclimation: (1) many metabolic systems may be effected at once; (2) these microenvironment modifications often have a shorter time constant than the induction of protein synthesis; and (3) given the high metabolic cost of protein synthesis, these microenvironment modifications are likely more economical. Lipid and pH changes have been examined with the hepatocyte preparation.

Changes in the lipid microenvironment of membrane-bound enzymes and the phenomenon of homeoviscous adaptation have been well documented, and Hazel (1984) has recently reviewed these processes in fish. Temperature and thermal history are also found to alter substrate incorporation into lipids (Hazel and Prosser 1979), total lipogenic capacity and the ratio of tritium incorporated into fatty acids compared with sterols (Hazel and Sellner 1979), fatty acid incorporation into phospholipids (Hazel 1982), and desaturation and elongation of fatty acids (Sellner and Hazel 1982). These studies, using rainbow trout hepatocytes, support those previously in the literature on adaptation.

The pH of blood and many intracellular compartments increases with decreasing acclimation temperature in a variety of ectothermic species, including fish (Reeves 1977; Walsh and Moon 1982; Cameron 1984). The slope of this relationship is approximately -0.017 pH unit$\cdot$°C^{-1}; fish blood and tissue pH at 5°C is about 0.4 unit more alkaline than at 30°C. This change in pH roughly parallels the change in the neutral pH of water with temperature, and an organism that allows its blood or tissue pH to change in this manner maintains a constant fractional dissociation state (or alpha) of the amino acid residues of its proteins. Hence, this strategy of acid–base regulation is termed "alphastat" regulation (Reeves 1977). Alphastat pH regulation leads to the preservation of many key functions of a variety of enzymes in spite of large changes in temperature (Somero 1981).

The importance of alphastat pH regulation to metabolic homeostasis during temperature changes depends upon how rapidly these pH adjustments occur. Blood pH adjustments are complete only 10–20 h after a 10° temperature change (Heisler 1978). Technical considerations make it difficult to monitor the response time of pHi to temperature changes in vivo. However, in vitro studies with American eel hepatocytes (Walsh and Moon 1983) have demonstrated that (1) pHi adjustments are rapid (in the order of minutes), (2) they can occur independent of systemic adjustments, (3) they occur at different rates during cooling and warming (Fig. 2), and (4) they mimic changes in vivo. Presently, hepatocytes are being used to determine the membrane transport and metabolic processes responsible for these temperature-induced changes in pHi (Walsh 1985).

Although it is apparent that alphastat pHi adjustments lead to a marked stabilization of many enzymic processes (Somero 1981), the role of these pHi changes as a metabolic regulatory signal during changes in temperature is still unclear. Previous studies of fish liver function have demonstrated that a preferred channelling of carbohydrate into the pentose shunt, at the expense of glycolysis, occurs at colder acclimation temperatures (Stone and Sidell 1981). A recent study of the sea raven hepatocyte system revealed that similar changes in carbohydrate metabolism could be rapidly induced (1–3 h) by cold exposure (Walsh et al. 1985). However, in this species, changes in pHi were minimal during these temperature changes, suggesting that other regulatory mechanisms are responsible for the observed changes in carbohydrate metabolism.

Perspectives

This review was not meant to be exhaustive, but instead, to examine studies that support the use of the fish hepatocyte preparation as a viable metabolic model. It is also meant to caution the new investigator so that methods used in the mammalian literature are not simply transferred to the fish studies. Metabolically, fish hepatocytes have provided a great deal of information on pathways including gluconeogenesis, lipogenesis, ketogenesis, and sterol, protein, and nucleic acid biosynthesis. Obviously not everything is known concerning any one pathway, but the basic groundwork has been established. The control of these pathways, and the effects of external factors (pH, temperature, hormones), are just now being studied using these preparations. The recent studies by Kent et al. (1983), Kent and Prosser (1984), and Koban et al. (1984) imply that the control of metabolic processes resides primarily within the liver cell, and may not be totally dependent upon complex systemic changes. This appears to be the case for temperature, and the generality of this finding needs to be assessed. Simon (1985) has used a modified digitonin fractionation procedure to establish the compartmentalization of ATP and ADP between mitochondria and cytosol of trout hepatocytes. Studies such as this will further elucidate the metabolic characteristics of the fish liver.

To date, most fish researchers have employed freshly isolated liver cells in suspension. A few recent publications have studied fish hepatocytes in primary culture with regard to protein synthesis (O'Grady et al. 1982; Haschemeyer and Mathews 1983; Kent and Prosser 1984) and steroid hormone binding sites (T. P. Mommsen and C. B. Lazier, unpubl. data). Too few studies are yet available to establish a basic medium for fish hepatocyte culture, although a modified Waymouth's medium has been used (Haschemeyer and Mathews 1983; T. P. Mommsen and C. B. Lazier, unpubl. data). The investigator must ensure compatibility of these media with the species used, just as mentioned with regard to hepatocyte isolation media. For example, plating of cells should be done on a collagen or matrix prepared from the species (Haschemeyer and Mathews 1983;

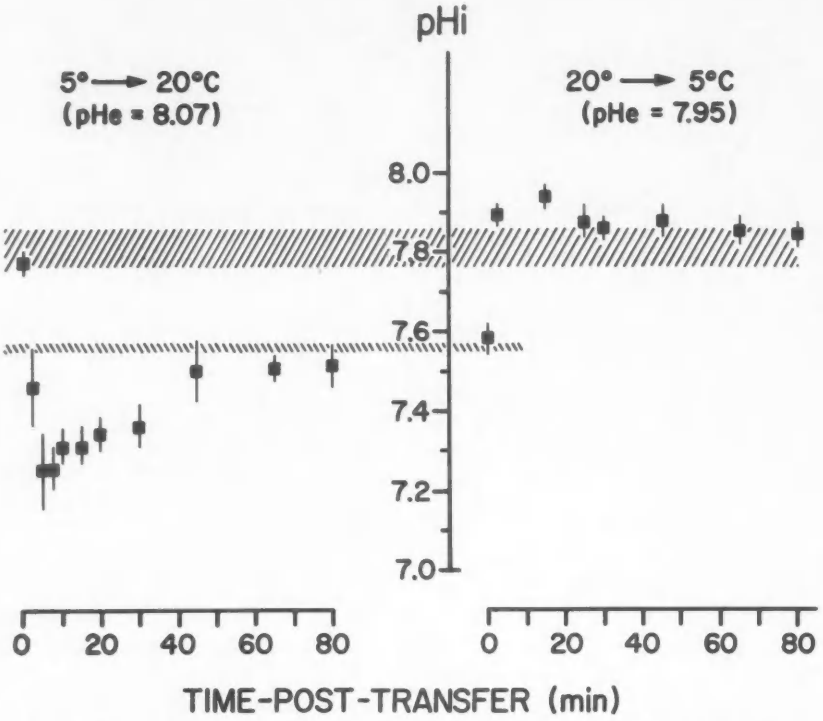

FIG. 2. Effect of acute temperature transfer on pHi in American eel hepatocytes. Cross-hatched areas represent steady-state (acclimated) pHi values (±SE, 5 to 7 experiments) in 5°C (upper) and 20°C (lower) hepatocytes. Taken from Walsh and Moon (1983).

Koban and Prosser 1983). The culture of fish hepatocytes may alleviate some of the variability observed in metabolic and hormone studies.

The use of fish hepatocyte cultures in the fields of pharmacology and environmental toxicology is particularly promising. Fish hepatocytes have been used to study the metabolism of xenobiotics (Parker et al. 1981; Anderson 1984) and for carcinogenicity testing (Bailey et al. 1984; Klaunig 1984). As stated earlier, the use of hepatocytes allows for multiple and simultaneous testing of compounds on cells derived from a single donor. This experimental approach obviates the use of elaborate control systems, for instance in the analysis of metallothionein induction after administration of variable amounts of heavy metals (Roch and McCarter 1984). The trout hepatocyte system has already been found to metabolize acetaminophen differently than mammals (Parker et al. 1981).

The interaction between temperature and xenobiotic susceptibility is an area worth mentioning. Thermal modulation of an organism's detoxification abilities may have profound effects on the outcome of toxicological studies, and it is imperative that the life history of the species is considered when such studies are designed and carried out.

The use of fish hepatocyte preparations in fisheries research is increasing rapidly. As its functioning has been found to this point to be consistent with the role of the liver in situ, its advantages as an experimental tool will facilitate its use in other basic and applied fisheries studies.

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Selenium Requirement of a Bloom-Forming Planktonic Alga from Softwater and Acidified Lakes

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Wehr, J. D., and L. M. Brown. 1985. Selenium requirement of a bloom-forming planktonic alga from softwater and acidified lakes. Can. J. Fish. Aquat. Sci. 42: 1783–1788.

Lakewide odor episodes have been associated with blooms of the planktonic alga *Chrysochromulina breviturrita* Nicholls (Prymnesiophyceae) in lakes influenced by acidic precipitation. We demonstrate an absolute requirement in this flagellate for selenium (Se) in axenic culture. The alga is capable of utilizing several forms of Se: selenite, selenate, dimethylselenide (DMSe), and selenomethionine. This is believed to be the first demonstration of the utilization of DMSe as a Se source by any organism. As the microflora of aquatic sediments recycle Se as volatile DMSe, this may have significance in its biogeochemistry in acidified waters. Bioassays of lake waters from the Muskoka–Haliburton region of Ontario show that in one lake (Dickie) a simulated Se spike resulted in a significant increase (70%, $p < 0.05$) in algal growth. Growth in Cinder Lake water, which had supported a population of *C. breviturrita*, was marginally stimulated (24%, $p < 0.05$). Distant coal-fired power plants, which have been implicated in the episodic deposition of Se, may account in part for the unpredictable blooms of the alga. *Chrysochromulina breviturrita* cannot be maintained in culture without this micronutrient, and several Se sources may be important triggering factors for the initiation of blooms.

Dans les lacs sous l'influence de précipitations acides, des périodes de présence d'odeurs ont été associées à des proliférations pléthoriques de l'algue planctonique *Chrysochromulina breviturrita* Nicholls (Prymnesiophyceae). Les auteurs démontrent qu'en culture axénique, le sélénium (Se) est absolument nécessaire à ce flagellé qui peut l'utiliser sous plusieurs formes : sélénite, séléniate, diméthylséléniure (DMSe) et sélénométhionine. On croit que ceci représente la première preuve de l'utilisation du DMSe par un organisme comme source de Se. Le fait que la microflore des sédiments lacustres recycle le Se sous la forme de DMSe volatile peut avoir une importance pour sa biogéochimie dans les eaux acides. Des analyses biologiques d'échantillons d'eau recueillis dans des lacs de la région Muskoka–Haliburton (Ontario) ont révélé que dans le lac Dickie, un important apport simulé de Se a entraîné une prolifération phytoplanctonique importante (70 %, $p < 0{,}05$). Dans le lac Cinder, habitat d'une population de *C. breviturrita*, la croissance n'a été qu'à peine stimulée (24 %, $p < 0{,}05$). Des centrales thermiques alimentées au charbon situées à distance, qui ont joué un rôle dans les dépôts épisodiques de Se, peuvent être en partie responsables des poussées phytoplanctoniques imprévisibles. *Chrysochromulina breviturrita* ne peut être gardé en culture sans cet oligoélément et plusieurs sources de Se peuvent être d'importants facteurs dans le déclenchement des efflorescences.

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Deposition of acids from SO_2-emitting sources have been identified as a primary cause of the acidification of softwater lakes in the eastern United States and Canada (Dillon et al. 1978; Loucks and Glass 1984). Anthropogenic emissions are estimated to account for more than 90% of the total SO_x emissions in eastern North America (Galloway and Whelpdale 1980), and principal sources are from coal combustion and metal smelting industries (Bubenick et al. 1983). In southern Ontario a number of lakes within a broad region of the Precambrian Shield now have reduced pH, which has been shown to be directly linked to anthropogenic SO_2 sources (LaZerte and Dillon 1984). In addition to depressed pH and alkalinities in affected lakes, metal mobilization (Schindler et al. 1980b; Wright et al. 1980) and deposition (Jeffries and Snyder 1981) have been observed. Observational and experimental studies of biological responses have documented declines in fish populations, simplification of plankton communities, and reductions in microbial numbers and rates of organic matter decomposition (Hendrey et al. 1976; Schindler and Turner 1982; Bubenick et al. 1983).

Another biological effect, which is believed to be linked with acidification, has recently been observed. Since 1978 softwater lakes in eastern North American have been affected by obnoxious ("rotten cabbage") lakewide odors. Odor episodes have all been associated with nearly unialgal blooms of the plankton alga *Chrysochromulina breviturrita* Nicholls (Nicholls et al. 1982). In Ontario, blooms have developed in moderately acidic (pH 5.5–6.2), poorly buffered (<160 μEq HCO_3^-/L) lakes in the Muskoka–Haliburton region (Nicholls et al. 1982), which is influenced by acid precipitation (Dillon et al. 1978; Scheider et al. 1979; LaZerte and Dillon 1984). In addition, following 2 yr of artificial acidification of Lake 302 (south basin) with H_2SO_4, a bloom of *Chrysochromulina* spp. (containing both *C. breviturrita* Nicholls and *C. laurentiana* Kling (Kling 1981)), with

odor, was observed (M. A. Turner, Freshwater Institute, Winnipeg, Man., pers. comm.). Recent experiments in our laboratory with an axenic culture of *C. breviturrita* isolated from a bloom in one of the affected lakes in Central Ontario have verified these field observations. A pH tolerance range of 4.0–6.9 was established and experiments have also demonstrated inhibition of growth as a result of HCO_3^- additions exceeding approximately 100 μEq/L (Wehr et al. 1985).

During the development of a chemically defined growth medium for *C. breviturrita* we were aware that earlier workers (Pintner and Provasoli 1968) were unable to maintain consistent growth of marine *Chrysochromulina* spp. without the addition of selenium (Se) as a trace element. Subsequently we also discovered that trace amounts of Se markedly improved the growth and long-term maintenance of *C. breviturrita*. Our interest in the role of Se was heightened, as it has been shown that the principal sources of SO_2 emission (coal combustion, metal smelting) also emit substantial quantities of Se (Andren et al. 1975; National Acadamy of Sciences Committee 1976; Nriagu and Wong 1983). In one instance, deposition from coal-fired power plants resulted in marked increases in Se in the water and biota of contaminated ponds (Gutenmann et al. 1976). Acidified lakes within a 30-km radius of Cu–Ni smelters in Sudbury, Ontario, were also Se enriched (Nriagu and Wong 1983).

Our objectives in this study were to (1) determine the degree of Se requirement by *C. breviturrita* in culture, (2) establish whether a dose–response relationship exists for Se, and (3) determine the potential for Se as a possible triggering factor in blooms of the alga in acidified lakes using bioassays.

Methods and Materials

Details of isolation and development of a chemically defined medium have been given previously (Wehr et al. 1985) and only a brief summary is given here. *Chrysochromulina breviturrita* was collected in July 1982 using surface water grab samples from Cinder Lake in Ontario (78°56′W, 45°04′N) during an odiferous bloom and subsequently isolated into unialgal, axenic culture. Due to the possibility of confusion of the identity of *C. breviturrita* with a recently described species, *C. laurentiana* Kling (Kling 1981), an exhaustive study of the scales of our isolate was carried out using transmission electron microscopy (TEM). Using shadow cast, negative staining, and freeze-etch methods with TEM on many occasions, it was determined that only *C. breviturrita* Nicholls emend Kling was present in the strain (No. BT130) used in this study (L. M. Brown, unpubl.).

The medium used was designed to approximate the low-alkalinity waters of acid-sensitive lakes in Ontario. The basal medium without added Se (Muskoka No. 20) contained (M = mol/L) 103 μM $CaSO_4 \cdot 2H_2O$, 44 μM $MgSO_4 \cdot 7H_2O$, 100 μM $NH_4H_2PO_4$, 3 μM $CaCl_2$, 14 μM KCl, 18 μM $FeSO_4 \cdot 7H_2O$, 8.1 μM H_3BO_3, and 1.2 μM $Na_2EDTA \cdot 2H_2O$. Trace metals included 4.5 μM $MnCl_2 \cdot 4H_2O$, 84 nM $CoCl_2 \cdot 6H_2$, 78 nM $CuSO_4$, 76 nM $ZnCl_2$, 50 nM $NaMoO_4$, and 19 nM $VOSO_4 \cdot 2H_2O$. The "f/2" vitamin mix (Nichols 1973), thiamine (0.3 μM), biotin (2.5 nM), and B_{12} (0.37 nM) was also added. Media were adjusted to pH 6.0 with NaOH (approximate total 30 μM), and a "Ross" semimicro combination electrode (Orion model 810300) was used to measure pH. Cultures were axenic and routinely tested for axenicity.

All glassware was acid washed (10% HNO_3, 12 h) and rinsed in deionized and distilled water prior to use. The water used for media and stock solutions was deionized and distilled. Media were routinely sterilized by autoclaving, except in treatments using different oxidation states of Se (H_2SeO_3, H_2SeO_4) and organic forms of Se. These latter treatments were filter sterilized (0.2-μm pore size). With one exception, experiments were run using 125-mL Ehrlenmeyer flasks with 50 mL of medium.

An experiment with volatile selenides (dimethyselenide (DMSe), dimethyldiselenide ($DMSe_2$)) and their controls used sealed 7-mL "funnel top" ampoules (Wheaton Glass) containing 3 mL of medium as follows. A container with 100 mL of Se-free medium was inoculated with *C. breviturrita* and then bubbled for 15 min with filtered (0.2-μm pore size) 5% CO_2 in air, which was mixed using a gas proportioner (Matheson Ltd.). Preliminary experiments indicated that CO_2 bubbling was necessary for growth in sealed containers. Aliquots (3 mL) of the inocula were added aseptically to sterile ampoules and sealed with sterile serum stoppers. Se was added using a 10-μL syringe and the ampoules were flame sealed. DMSe and $DMSe_2$ stocks were prepared in redistilled ethanol; hence, controls with inorganic Se (as H_2SeO_3) or no Se also had ethanol added. Suppliers of compounds were Baker Chemical (H_2SeO_3), Alfa Products (H_2SeO_4, DMSe, $DMSe_2$), and Sigma Chemical Company (seleno-DL-methionine, DL-methionine, selenocystamine dihydrochloride).

Growth assays were carried out using lake water samples collected 10 September 1984 from Cinder Lake (pH 6.40), the original source of the isolate of *C. breviturrita* used in the experiments, and from Dickie Lake (pH 6.36) (79°05′W, 45°05′N), another softwater lake in the Muskoka region of Ontario. Waters were prefiltered (0.45-μm pore size) and supplemented with either the f/2 vitamin mix ("control"), 100 μM $NH_4H_2PO_4$ plus vitamins, 0.63 μM H_2SeO_3 plus vitamins, or all three additions. These enriched lake waters were adjusted to pH 6.0, autoclaved, and inoculated as with the chemically defined medium.

Unfiltered samples of Dickie and Cinder lake waters were also examined for the presence of *C. breviturrita* using phase-contrast microscopy. Counts of the dominant algal taxa were carried out using Sedgwick–Rafter cells after treatment with Lugol's iodine. The total alkalinities (by titration with standardized 0.010 M H_2SO_4) of fresh samples of Cinder and Dickie lake waters were determined to be 36 and 14 μEq/L, respectively. During 1983, concentrations of total N and total P average approximately 480 μg N/L and 15 μg P/L, respectively, in these lakes (K. H. Nicholls, Ontario Ministry of the Environment, Rexdale, Ont., pers. comm.). An inspection of the plankton from the unfiltered water samples revealed the presence of a moderately large (approximately 250 cells/mL) population of *C. breviturrita* in Cinder Lake only. Otherwise, the phytoplankton communities were structurally similar at the time of sampling.

To maintain *C. breviturrita* for more than two or three transfers it was necessary for stock cultures to be grown in a medium supplemented with Se (0.63 μM). Therefore, prior to experiments cells were starved of Se in the following manner. In late exponential phase (between days 8 and 12), healthy cells remained in a film at the water surface. The medium was aspirated from the bottom and the majority of liquid (90–95%) removed aseptically from the cells. Cells were then inoculated into a large volume of Se-free medium (e.g. 800 mL) for a period of at least 5 but no more than 10 d. Starvation periods exceeding this generally yielded unhealthy inocula or even cell lysis. After the starvation period enough medium was aspirated

TABLE 1. Comparison of growth of *C. breviturrita* in various media relative to enriched Cinder Lake water. Lake water was collected July 1982 during a bloom (enrichment = 100 μM $NH_4H_2PO_4$ and f/2 vitamins; M20 = Muskoka No. 20 (see text); yeast extract concentration = 0.5 g/L; added Al concentration = 100 µg/L; added Se concentration = 50 µg/L).

Medium	Maximum growth rate (divisions/day)	Yield (10^5 cells/mL)
Enriched Cinder Lake	0.70±0.14	3.09±0.32
M20 (basal medium without Se)	0.65±0.07	0.30±0.05
M20 + yeast extract	0.68±0.17	3.04±0.25
M20 + Al	0.59±0.10	0.23±0.10
M20 + Se^{IV}	0.78±0.10	2.65±0.30

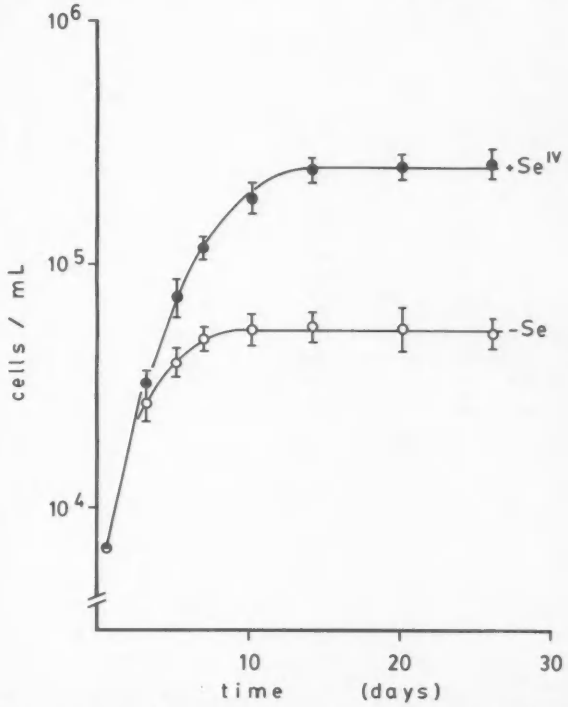

FIG. 1. Comparisons of growth of *C. breviturrita* in a defined medium with and without Se added as H_2SeO_3 (+Se = 50 µg Se/L (0.63 μM), $n = 3$, $\bar{X} \pm$ SD).

away to produce an inoculum whose density was approximately 1×10^5 to 2×10^5 cells/mL. Experimental regimes (flasks or ampoules) were inoculated to produce an initial density of 2×10^3 to 5×10^3 cells/mL.

Experiments were run at 20°C with a subsurface irradiance of 60–70 $\mu E \cdot m^{-2} \cdot s^{-1}$ ("cool white" fluorescent lights) on a 12 h light : 12 h dark cycle. Cells were harvested (3 mL) aseptically for growth estimates. Growth was measured by increases in cell number. Counting was carried out using a Bio/Physics (model 6300 A) Cytograf laser-based particle counter (Ortho Instruments Division, Westwood, MA). Counts were calibrated using cell suspensions of known densities from hemacytometer counts. Experiments were run in triplicate and usually repeated twice.

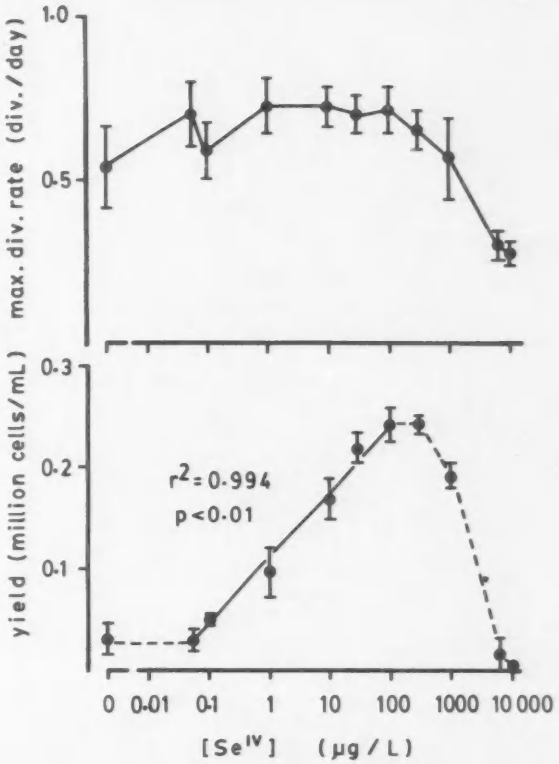

FIG. 2. Effect of Se^{IV} (as H_2SeO_3) concentrations on maximum division rate and cell yield of *C. breviturrita* in a defined medium. Solid line on yield plot fitted by linear regression ($n = 3$ for 0–0.1, 500 – 10 000 µg Se/L; $n = 6$ for 1.0, 10–100 µg Se/L; $\bar{X} \pm$ SD).

Results

The development of a chemically defined growth medium for *C. breviturrita* led to some comparisons between growth in synthetic media, partially defined media, and enriched lake water (Table 1). One chemically defined medium, Muskoka No. 20, would only support vigorous growth ($>10^5$ cells/mL at stationary phase) for one or two subcultures after maintenance in enriched Cinder Lake water; yeast extract or selenite yielded vigorous, reproducible growth comparable with enriched Cinder Lake water (Table 1). Because yeast extract rendered the medium undefined, its use was not continued. Typical growth curves demonstrated that the cell yield was significantly (ANOVA, $p < 0.05$) increased in a defined medium with selenite (Fig. 1). This enhancement ranged from 5- to 10-fold depending on the degree of Se starvation or carryover. Maximum division rates in the two treatments (days 0–3) were not significantly different ($p < 0.05$). Further subcultures in the absence of Se resulted in virtually no growth and, ultimately, cell lysis.

A dose–response of *C. breviturrita* growth over a broad range (greater than six orders of magnitude) of Se (as selenite) concentrations (Fig. 2) indicated a minimum stimulation of cell yield in culture at 0.1 µg/L (doubled yield). The growth response between 0.05 and 100 µg/L had a significant relationship to $\log_{10}$ Se concentration ($p < 0.01$). The apparent optimum Se concentration range in this medium was 50–500 µg/L. Above

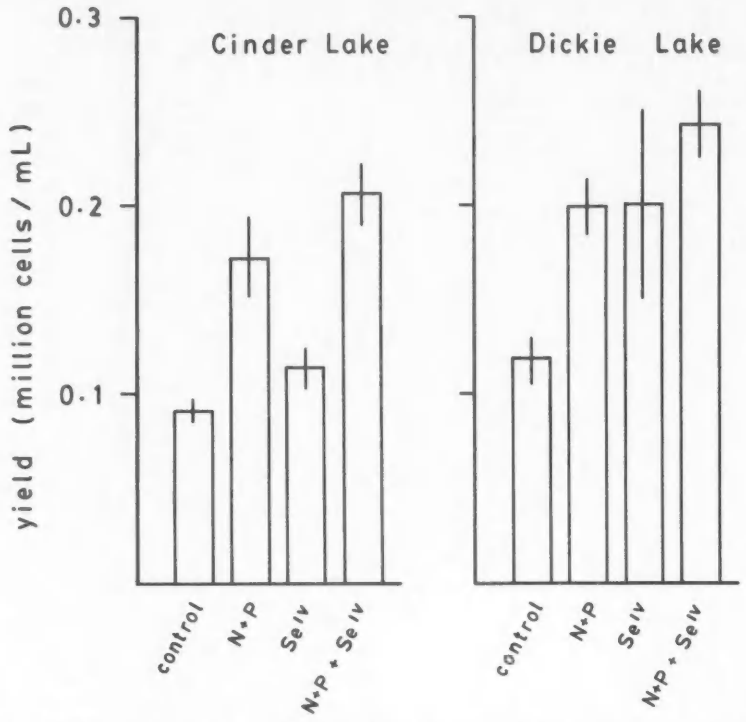

FIG. 3. Growth responses of *C. breviturrita* to Se and/or nutrient additions in assay water collected (10 September 1984) from Cinder and Dickie lakes of the Muskoka–Haliburton region of Ontario (control = f/2 vitamins only; N + P = 100 μM $NH_4H_2PO_4$; Se^{IV} = 0.6 μM H_2SeO_3 (50 μg Se/L); N + P + Se^{IV} = both additions; $n = 3$, $\bar{X} \pm$ SD).

these concentrations, growth was increasingly inhibited. Maximum division rates (Fig. 2) indicate no significant differences over nearly the entire range, except a reduction at ≥5 mg Se/L, where marked inhibition of cell yield was also observed.

As growth in enriched lake water that had been collected during a bloom (July 1982) proved to be successful, assays were conducted using water from two softwater lakes in which *C. breviturrita* has been observed previously, but during a "non-bloom" period: Cinder Lake and Dickie Lake. Growth of *C. breviturrita* was significantly stimulated relative to controls ($p < 0.05$) in both lake waters by the addition of N + P (Fig. 3). In Cinder Lake the addition of Se alone had a small but significant effect ($p < 0.05$), but yield in Dickie Lake was increased by 70% ($p \ll 0.05$). Growth in Dickie Lake water was also greater in the treatment with Se and N + P (2.42×10^5 cells/mL) than N + P alone (1.99×10^5 cells/mL).

Both selenite (Se^{IV}, as H_2SeO_3) or selenate (Se^{VI}, as H_2SeO_4) added to the chemically defined medium resulted in a similar stimulation relative to −Se controls (Table 2). Because an apparent stimulation may be due to inorganic Se alleviating phosphate toxicity (Broyer et al. 1972), an experiment was conducted over decreasing PO_4^{3-} concentrations (Table 3). There was no Se–PO_4^{3-} interaction, as a 10-fold decrease in PO_4^{3-} (from 100 to 10 μM) had no significant effect ($p > 0.05$) on growth of *C. breviturrita* in the presence or absence of Se. Sulfur–selenium antagonism was also considered, using excess concentrations (10-fold increase) of SO_4^{2-} in the presence and absence of Se (Table 3). No significant increase was observed when S replaced Se, nor did excess SO_4^{2-} affect yield in the presence of Se.

TABLE 2. Effects of Se oxidation state on growth of *C. breviturrita*, as maximum division rate and cell yield, in a defined medium (Se^{IV} = selenite as H_2SeO_3; Se^{VI} = selenate as H_2SeO_4; both +Se treatments 50 μg Se/L; $n = 6$; $\bar{X} \pm$ SD; * value significantly different from −Se control at $p < 0.05$).

Se treatment	Maximum growth rate (divisions/day)	Yield (10^5 cells/mL)
−Se	0.65±0.10	0.40±0.14
Se^{IV}	0.70±0.11	2.26±0.30*
Se^{VI}	0.78±0.05	2.32±0.24*

The possible stimulatory effects of organic Se compounds (equivalent Se concentrations: 50 μg/L) were also examined (Fig. 4). Significant positive effects were found for chemically unrelated compounds; selenite (H_2SeO_3), DMSe, and DL-selenomethionine. The S-containing analogue of the latter compound did not stimulate growth of *C. breviturrita* ($p > 0.05$) compared with the −Se control. Results were ambiguous with the addition of selenocystamine, and a significant inhibition of growth ($p < 0.05$) was observed with $DMSe_2$.

TABLE 3. Tests for interactions of Se^{IV} (as H_2SeO_3) with S (as $CaSO_4$) and with P (as NaH_2PO_4), using two concentrations of SO_4^{2-} and three concentrations of PO_4^{3-}, on cell yields of *C. breviturrita* (+Se = 50 μg/L; $n = 3$; $\bar{X} \pm$ SD).

Potential interfering ion	Concentration (μ*M*)	Se^{IV}	Yield (10^5 cells/mL)
SO_4^{2-}	100	+	3.03±0.13
		−	0.25±0.05
	1000	+	2.89±0.38
		−	0.26±0.01
PO_4^{3-}	10	+	2.20±0.22
		−	0.30±0.06
	50	+	2.36±0.17
		−	0.28±0.05
	100	+	2.38±0.20
		−	0.26±0.03

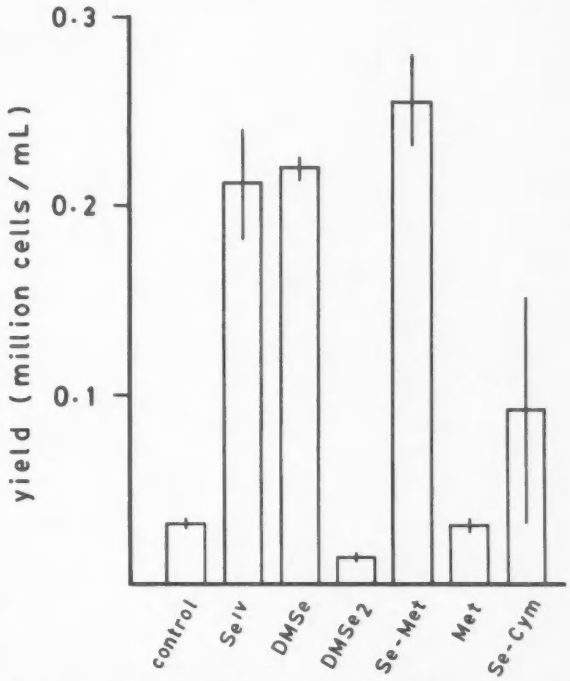

FIG. 4. Growth responses of *C. breviturrita* (as cell yield) to organic Se compounds relative to controls and inorganic Se^{IV} addition (all Se concentrations 50 μg Se/L; Se^{IV} = selenite as H_2SeO_3; DMSe = dimethylselenide; $DMSe_2$ = dimethyldiselenide; Se-Met = seleno-DL-methionine; Met = DL-methionine without Se equimolar to Se-Met; Se-Cym = selenocystamine; $n = 3$, $\bar{X} \pm$ SD).

Discussion

Physiological studies on marine *Chrysochromulina* spp. demonstrated an apparent requirement for Se, although dose–response relationships were erratic (Pintner and Provasoli 1968). Experiments with *C. breviturrita* clearly demonstrated a significant growth response relative to Se^{IV} concentrations, spanning more than three orders of magnitude (0.05–100 μg Se/L). A similar dose–response to Se^{IV} has been reported for the dinoflagellate *Peridinium cinctum* and the diatom *Stephanodiscus hantzschii*, from circumneutral to slightly alkaline lakes (Lindström and Rodhe 1978; Lindström 1983). However, unlike *C. breviturrita*, neither species responded positively to Se^{VI}. The dose–response was suggested as evidence of a possible bioassay of "available" Se (Lindström 1980).

One of the definitive methods for determining if Se is an essential micronutrient is that of establishing whether growth is inhibited if the element is omitted (Anderson and Scarf 1983). This is satisfied for *C. breviturrita* (Tables 1–3; Fig. 1, 2). We have ruled out Se antagonism (Table 3) of phosphate toxicity, as shown in some higher plants (e.g. Broyer et al. 1972), and SO_4^{2-} interaction or antagonism (Schrift 1954; Wheeler et al. 1982). Stimulation of *C. breviturrita* growth by several chemically unrelated sources of Se, Se^{IV}, Se^{VI}, selenomethionine, and DMSe further suggests that there is a true requirement for Se which may perform some specific enzymatic function, such as in glutathione peroxidase in mammalian systems and formate dehydrogenase in bacterial systems (Stadtman 1979).

Assays of Dickie lake water suggest that when tested, Se may be limiting growth of *C. breviturrita* and could be a possible triggering factor for blooms. This is in addition to the stimulation by N and P, as would be expected in an oligotrophic lake. Growth in Cinder Lake, which had supported a sizable population of *C. breviturrita* in situ, was marginally but significantly stimulated (24%, $p < 0.05$) by the Se addition. Although concentrations of total Se in freshwaters rarely exceed 0.1 μg/L (1.3 n*M*) (Lindström 1983; Nriagu and Wong 1983), cell densities of blooms in affected lakes are roughly two orders of magnitude less than in culture (approximately 3×10^3 vs. 3×10^5 cells/mL). Concentrations of Se^{IV} or Se^{VI} necessary to stimulate growth in situ or in continuous culture may be lower than observed in the laboratory thus far.

Dissolved organic Se fractions (e.g. amino acids), which are also utilized by the alga, are important in the biogeochemistry of the element in marine and fresh waters (Cutter and Bruland 1984; Takayanagi and Wong 1984). Although similar growth stimulation by selenomethionine has been demonstrated with other algae (Lindström 1983), we believe this may be the first demonstration of utilization of DMSe as a Se source. As a majority of Se in acidified waters may be deposited in sediments (Schindler et al. 1980a; Nriagu and Wong 1983), it is interesting to note that the microflora and aquatic sediments are capable of recycling Se as DMSe and other odor-producing forms (Chau et al. 1976).

One additional characteristic of the nearly unialgal blooms of *C. breviturrita* is its abrupt and unpredictable occurrence (Nicholls et al. 1982). Although atmospheric SO_2 and Se emissions are fairly ubiquitous in eastern North America (Andren et al. 1975; Furr et al. 1977), a systematic, daily study of aerosols identified a distinctly episodic nature to the transport of Se (along with SO_4^{2-} and As), which was linked to distant coal-fired power plants (Parekh and Husain 1981). Such short-term pulses of Se, while potentially stimulatory, may not be detected in routine surveys of aqueous trace elements. In addition to recycled organic Se from sediments, these atmospheric pulses may also prove to stimulate populations in lakes influenced by acid precipitation.

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Spring Meltwater Mixing in Small Arctic Lakes

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Bergmann, M. A., and H. E. Welch. 1985. Spring meltwater mixing in small arctic lakes. Can. J. Fish. Aquat. Sci. 42: 1789–1798.

Meltwater mixing in small arctic lakes at Saqvaqjuac (63°68′N, 90°40′W) was studied in 1980 and 1981 to evaluate the applicability of theoretical lake water renewal times to the modeling of ice-covered lakes. Two 370-GBq tritium additions were made to 7.09-ha P & N Lake. One was mixed with the unfrozen water at the time of maximum lake-ice thickness (May 1980) and the other was mixed with the lake immediately after freezing (October 1980). Dye experiments were also performed at four lakes to define the spatial and temporal distribution of the inflow and icemelt layers. Results from the tritiated water and dye addition experiments, as well as conductance and temperature profiles, showed that during ice-on, the cold low-density meltwater floated in a thin layer 0–100 cm beneath the ice, extended over the entire subice-surface area, and left the lake without mixing with the heavier subice water. These results imply that (1) lake models incorporating a lake flushing rate term need to be reevaluated to accommodate the lack of meltwater mixing beneath spring ice and (2) more attention should be given to the early spring meltwater chemistry and its distribution within the upper lake strata.

En 1980 et 1981, les auteurs ont étudié le mélange des eaux de fonte dans de petits lacs arctiques à Saqvaqjuac (63° 68′ N, 90° 40′ W) en vue d'évaluer l'applicabilité de temps théoriques de renouvellement des eaux lacustres à la modélisation de lacs recouverts de glace. Deux apports de GBq tritium 370 ont été effectués sur 7,09 ha des lacs P et N, dont un a été mélangé à l'eau non gelée quand la glace était à son épaisseur maximale (mai 1980) et l'autre, avec le lac immédiatement après le gel (octobre 1980). Les auteurs ont aussi mené des expériences avec des matières colorantes dans quatre lacs afin de déterminer la distribution spatiale et temporelle des couches d'eau de fonte et d'entrée des eaux. Les résultats obtenus des expériences sur l'eau ajoutée de tritium et sur l'apport de matières colorantes ainsi que les profils de la conductance et de la température ont révélé que, sous la couverture de glace, l'eau froide de fonte de faible densité flottait sous la glace en couche dont l'épaisseur variait de 0 à 100 cm, couvrait toute la superficie sous la glace et quittait le lac sans se mélanger avec l'eau plus dense. Ces résultats donnent à entendre que (1) les modèles descriptifs de lac englobant un taux d'élimination des eaux lacustres doivent être réévalués afin d'inclure le mélange des eaux de fonte sous la glace au printemps et (2) plus d'attention doit être portée à la chimie de l'eau de fonte au début du printemps et à sa distribution dans les strates lacustres supérieures.

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The theoretical turnover time (TT) of water in a lake is an important term in management models (Vollenweider 1969; Dillon and Rigler 1974), and assumes that all inputs mix completely with the lake:

$$(1)\quad TT = \frac{\text{lake volume } (m^3)}{\text{annual outflow volume } (m^3 \cdot yr^{-1})}.$$

However, results from a number of studies indicate that in ice-covered lakes, complete mixing probably does not occur, but rather that inflows can proceed directly to the outflow, mixing only with the upper layers of unfrozen water (Gröterud 1972; Schindler et al. 1974; Henrikson and Wright 1977; Hultberg 1977; Prentki et al. 1979; Hendrey et al. 1980). The purpose of this study was to make direct measurements of spring inflow and retention of icemelt and snowmelt by ice-covered lakes; thereby we refine estimates of lake turnover time and improve our ability to address limnological problems concerned with acid and nutrient loading as well as metal accumulation.

We did our experiments in conjunction with whole-lake nutrient addition experiments at Saqvaqjuac, a Canadian Department of Fisheries and Oceans research camp on Hudson Bay at 63°68′N, 90°40′W (Welch 1985). In P & N Lake, tritiated water was used as a tracer in a two-stage experiment, with the unfrozen water labelled in 1980 and both ice and unfrozen water labelled in 1981. In other experiments, Rhodamine B was added to melting ice surfaces and to inflows to determine the spatial pattern of stratification in a series of local lakes.

A lake was assumed to consist of three compartments: ice, subice (= unfrozen water below the ice at the onset of melt), and throughflow (= all inputs to the lake which were subsequently discharged via the outflow, after an unknown amount of mixing).

The traditional theoretical turnover time model (1) is based entirely on a lake's hydrological budget, hereafter called the

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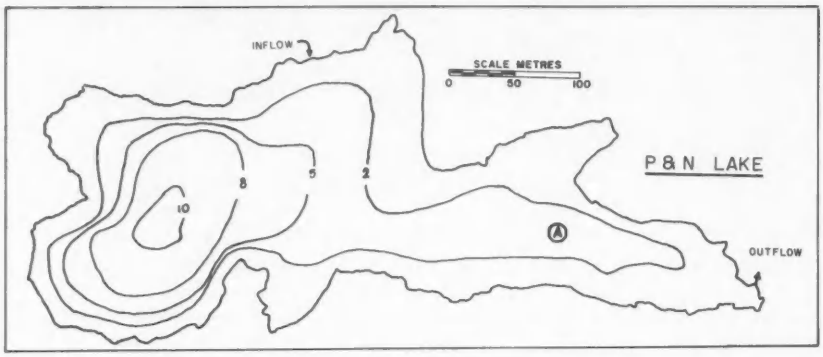

FIG. 1. P & N Lake. "Inflow" refers to a small temporary inflow used in a Rhodamine B dye addition (see Fig. 4 and 5). "A" is the location of the profiles reported in Fig. 3. Mean depth = 3.3 m; maximum depth = 10.2 m (Welch 1985).

H_2O dataset. This model assumes that each of the above compartments has the same turnover time as the whole lake. In this study two other whole-lake mixing models (M1 and M2) were derived, and the tritium data were used to evaluate the three models. The tritium data were developed by integrating the lake morphometry with the lake water inputs and outputs to form the *^{3}HOH dataset*. One model (M1) determined a theoretical turnover time (TT_c) for each of the three compartments, whereas a second model (M2) considered daily changes in the lake system and predicted outflow losses for the labelled watermass during the ice-on period.

To provide an independent comparison of the traditional model predictions and observed data, conductivity data were used in conjunction with the water budget data to predict daily outflow conductivity concentrations, which were then compared with the measured data.

Materials and Methods

For a description of the experimental area, lakes and morphometry, climate, and biology see Welch (1985). Saqvaqjuac lakes freeze about 1 October and ice thickness averages 1.7–1.9 m by May. Annual precipitation (mean for 1977–81) is 298 mm. Spring melt begins by late May or early June when ambient temperatures are near 0°C, although freezing of inflows and outflows sometimes occurs during this period and may last several days. With increasing air and water temperature a moat forms and extends to a maximum depth of approximately 1.5–2.0 m before the ice-pan begins its rapid deterioration. Lake water temperature is always >1°C by the beginning of melt, and increases to 4°C by iceout.

P & N Lake (Fig. 1 and Welch 1985), the site of the whole-lake ^{3}HOH experiments, is a headwater lake with a mean annual (1977–80) TT of 2.2 yr. Complete water flow data for P & N Lake for 1980 and 1981 were provided by Dalton (1981) and C. Allan and G. K. McCullough (Freshwater Institute, Winnipeg, Man., pers. comm.). Spring, Hawk, and Turkey lakes (Welch 1985), used in dye experiments, have TT's of 1.1, 2.9, and 0.4 yr, respectively.

Whole-Lake ^{3}HOH Addition Experiments

The first addition of ^{3}HOH to P & N Lake was on 16 May 1980, at the point of maximum water depth (Z_{max}). An amount of 370 GBq (10 Ci) contained in a vial (New England Nuclear) was added 0.3 m below the bottom of the ice in a stream of water pumped horizontally at $>100\,L\cdot min^{-1}$. A combination of compressed air and pumped water was then used to circulate the lake over the next 3 d, after which ^{3}HOH was completely mixed throughout the lake (19 May). Thermal stratification was reestablished by 22 May.

On 3 October 1980, another 370 GBq of ^{3}HOH was added to P & N Lake under 13 cm of ice. The vial was crushed, diluted in 200 L of lake water, and siphoned into the lake beneath the ice at $10\,mL\cdot s^{-1}$. Compressed air and an outboard motor were then used to completely mix the ^{3}HOH throughout the lake by 9 October.

Sample and Analytical Methods

Duplicate outflow samples from P & N Lake were taken at least daily over the entire flow periods of 1980 and 1981. To determine the stratification of meltwater beneath the ice, a suction sampler (Welch and Bergmann 1985) which drew water ($25\,mL\cdot s^{-1}$) from a layer about 1 cm thick was used to sample water through grids of holes. A 2-L Van Dorn water bottle was used to sample the lake during ice-off period. Ice samples were taken in 1981 on three occasions during the ice-on period (October–July) and sectioned into 2-cm intervals to determine the vertical distribution of ^{3}HOH in the ice.

Conductance (microsiemens per centimetre at 25°C) was measured using a Radiometer conductivity meter calibrated against a standard 0.01 mol $KCl\cdot L^{-1}$ solution, with a precision of ±5%.

The activity of ^{3}H (disintegrations per minute per cubic centimetre) was determined on 8-mL samples in liquid scintillation vials containing a 10-mL aliquot of fluor (Beckman All-Purpose scintillation cocktail). A scintillation counter (Beckman LS100) with an efficiency for tritium of 23 ± 3% (glass vials) and 21 ± 3% (plastic vials) was used to determine the activity of each sample (counting efficiency did not change throughout the study period). The samples were counted to 5% (2σ) statistical counting error. Including sample volume error, the precision of each ^{3}HOH analysis was ±8%.

In addition to routine monitoring of ice thickness and moat width (the distance between lake edge and ice-pan) for 1980 and 1981, in May 1981, marked rods (3 mm × 90 cm) were individually mounted on a 9-cm^2 clear plastic base and placed in 10-cm-diameter holes, with the bases resting on the ice in the

bottoms of the holes 40–80 cm deep. The holes were filled with water and left to freeze, leaving the rods extending above the ice surface. After the beginning of melt, the increase in rod lengths above the ice surface was used to measure the surface icemelt.

Lake temperature (T) measurements were made regularly from May 1980 to August 1981 using two different systems, a Hydrolab model RT-125 (±0.1°C) thermistor and a bead thermistor connected to an ohmmeter (±0.03°C).

Dye Methods

On 27 May and 4 June 1980 and 12 June 1981, 20 L of Rhodamine B dye solution was added over 0.5 h to P & N, Spring, and Hawk Lake inflows, respectively, to determine the paths of the inflowing water through the main lake body beneath the ice. Rhodamine B was also sprayed on a 12-m-diameter circle of candled Turkey Lake ice on 16 June 1980 to follow icemelt as it entered the lake. Rhodamine B fluorescence was measured in a Turner model III fluorometer.

Corrections for Losses of ^{3}HOH to the Sediments and Atmosphere

Losses of ^{3}HOH from the lake water to the sediments were determined by measurement of tritium profiles in the sediment porewater at 9.5 m depth in P & N Lake. The difference between the ^{3}HOH mass predicted for the whole lake from the water budget and that actually measured (both corrected for sediment loss) during the ice-off period was assumed to be due to ^{3}HOH loss from the lake surface via vapor exchange.[2] These losses were used together with the other measured losses to correct the ^{3}HOH mass balance.

Mass Calculation for ^{3}HOH

Mass (M) of ^{3}HOH is determined by

$$(2)\quad M = \int_{s=0}^{s=\max} A_s C_s d_s + \int_{i=0}^{i=\max} A_i C_i d_i$$

where A is the area of the midpoint of each contour interval and C is the tritium activity at depth for the subice (s) and ice (i) mass.

Models

In P & N Lake the volume of labelled subice water lost by the outflow during 1980 was determined by dividing the outflow ^{3}HOH mass by the corrected subice ^{3}HOH activity (corrected for sediment and gross evaporation losses). In 1981, when both ice and subice were labelled, the following equation was used:

$$(3)\quad V = T_O \Big/ \frac{T_S + T_I}{V_S + V_I}$$

where V = volume of labelled subice and ice lost via outflow (cubic metres), T_O = mass of ^{3}HOH lost via outflow (dpm) measured, T_S = subice mass of ^{3}HOH (dpm) at time of maximum ice thickness, V_S = subice volume (cubic metres) at time of maximum ice thickness, T_I = ice mass of ^{3}HOH (dpm) at time of maximum ice thickness, and V_I = ice volume (cubic metres, water equivalents) at time of maximum ice thickness.

Model M1 predicts annual turnover time (TT_i, years) as

$$(4)\quad TT_i = \frac{VW_i}{\sum_{i=1}^{3} VW_i} \times V_L \Big/ \frac{VH_i}{\sum_{i=1}^{3} VH_i} \times VO_i$$

where VW_i = mean annual volume of compartment i (cubic metres) as determined from the water budget, V_L = mean annual volume of lake (232 700 m^3), VH_i = mean annual volume (cubic metres) of compartment i as determined from pooled ^{3}HOH and H_2O datasets, and VO_i = mean annual volume of compartment i lost to the outflow (cubic metres).

For model M1, the ^{3}HOH and H_2O datasets for 1980 and 1981 were combined with the assumption that the proportion of outflow volume of each lake compartment did not change from one year to the next. From these pooled data it was possible to obtain estimates (cubic metres) for the proportion of each compartment of the total outflow volume.

Daily ^{3}HOH losses from P & N Lake outflow provided estimates of the losses (cubic metres) from the labelled subice compartment (1980) or subice/ice compartments (1981). In order to compare these results with those predicted by the traditional model (M1), a second model was developed to predict daily ^{3}HOH losses from the labelled compartment(s) (V_p, cubic metres).

Model M2 predicted V_p as

$$(5)\quad V_p = PT_O \Big/ \frac{PT_S + PT_I}{PV_S + PV_I}$$

where PT_O = predicted ^{3}HOH of outflow (dpm) and is given by

$$PT_O = \frac{(M_L + M_I - M_O)}{(V_L + V_i + V_I - V_O)} \times V_O$$

and PT_S = predicted ^{3}HOH of subice (dpm), PV_S = predicted volume of subice (cubic metres), PT_I = predicted ^{3}HOH of ice (dpm), 1981 only, PV_I = predicted volume of ice (cubic metres), 1981 only, M_L = total lake ^{3}HOH of previous day (dpm), M_I = mass of ^{3}HOH melted from ice (dpm), 1981 only, M_O = mass of ^{3}HOH lost via outflow (dpm), V_L = total lake volume of previous day (cubic metres), V_i = inflow volume (cubic metres), V_I = ice volume (cubic metres, water equivalent), 1981 only, and V_O = outflow volume (cubic metres).

To provide an independent comparison of the losses predicted by the traditional model and the observed data, measured conductance was compared with those estimates obtained using the water budget data on a daily basis.

Results

Profiles

The shape of the conductivity and temperature profiles during the melt period were similar in 1980 and 1981 (Fig. 2), but the tritium profiles differed. Profiles showed stratification, with a thin layer of less dense, colder water with lower salt concentration, located just below the ice. As springmelt continued, both the icemelt and snowmelt runoff layers beneath the ice-pan became thicker, with only a small encroachment into the deeper layer, as shown by the ^{3}HOH data (Fig. 3). Simultaneously, a shallow moat, which remained completely mixed, as determined by ^{3}HOH and conductance measurements (Bergmann 1982), formed between the ice-pan and the lake edge (not shown).

Dye Experiments

Four dye experiments, one ice and three inflow additions,

[2]Analysis of the ^{3}HOH losses to the sediments and to evaporation will be discussed in a subsequent paper.

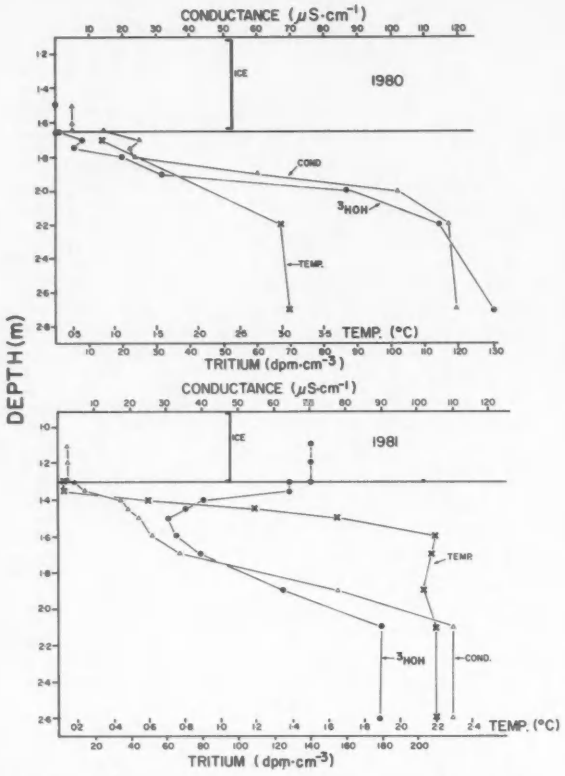

FIG. 2. Profiles of conductance, temperature, and tritium activity in P & N Lake, 5 June 1980 and 10 June 1981.

showed the flow pattern of subice spring meltwater. All inflow Rhodamine B additions provided similar results; the dye extended from the inflow in a thin plume beneath the ice and became wider and deeper before reaching the outflows.

At P & N Lake (27 May – 6 June 1980) the inflow rate was about 5 $L \cdot s^{-1}$ and the dye was carried out in all directions from the source (Fig. 4), forming a thin layer beneath the ice and becoming deeper over the duration of the experiment (Fig. 5).

The initial dye distribution from Spring Lake (4–7 June 1980) inflow (about 20 $L \cdot s^{-1}$) was in a narrow band, located a few centimetres below the bottom of the ice (Fig. 6). The dye reached the outflow 4 d after the addition, with the highest concentrations in the lake still just below the ice.

Dye was added to Hawk Lake inflow (12–14 June 1981) at peak flow (about 50 $L \cdot s^{-1}$) and was found at the outflow after 33 h. Highest Rhodamine B concentrations were again found a few centimetres beneath the ice (Fig. 7).

These three experiments indicate that the meltwater inflows (1) proceed toward the outflows as vertically discrete masses, (2) tend to concentrate a few centimetres *below* the subice surface rather than immediately adjacent to it, and (3) tend to mix more vertically as inflow volume increases.

The addition of dye to the candled ice surface of Turkey Lake (17 June 1980) illustrated that the dye moved down through the porous ice, and that the highest concentrations were found contiguous to the ice (Fig. 8). Because the amount of dye added was small, the dilution was rapid and no dye was found >3 m from the addition site. Thirty-six hours after addition, the dye was no longer detectable. This experiment showed that the icemelt water remains immediately below the ice.

General Flow Pattern

During the melt period in P & N Lake, therefore, there were three distinct layers beneath the ice: icemelt on top, followed by inflowing snowmelt from runoff, and subice lakewater on the bottom. Phosphorus and silica profiles for P & N Lake (unpubl.) showed that inflowing particulate and dissolved substances also conformed to this stratification. As icemelt and runoff continued, the meltwater layer became thicker, with a small amount of mixing with the subice water. This limited vertical mixing persisted until ice-off, which was also the time of spring turnover, and is typical of arctic lakes (Schindler et al. 1974; Minns 1977; LaPerriere 1981).

Meteorological conditions interacted with lake morphometry to modify the subice flow pattern. The dye addition to the P & N Lake inflow took place during a period of early flow. Shortly after the dye addition a period of cold weather caused the inflow and outflows to begin freezing, and 24 h after addition the flow stopped. The dye slowly moved throughout a large portion of the lake, the dye still just below the ice, and the energy required to distribute the dye can probably be attributed to the residual water movement set up by the inflows and density differences within the lake. When warmer weather caused flow to resume 4 d later, the dye had become widely dispersed throughout the upper 1 m beneath the ice, and with the subsequent decrease in concentration below the threshold of detection, a further description of dye movement was impossible.

The Spring Lake and Hawk Lake inflow experiments took place during periods of constant flow which resulted in a faster movement of dye to the outflow. The pattern of dye distribution was somewhat different for the Hawk Lake experiment (12–14 June 1981) from that found for Spring Lake (4–7 June 1980), due to the greater volume of Hawk Lake's inflow. The dyed inflow water was initially found between 10 and 40 cm beneath the bottom of the ice, in a direct band to the outflow. The fastest moving water, the topmost layer, was characterized by the rapid dilution and then total replacement of the dye in the layer 20–30 cm below the ice, while the slower water was found between 0 and 20 cm and 30 and 60 cm below the ice and had a longer residence time. This indicated that the inflowing water (throughflow compartment) had the greatest velocity in the water column and therefore the shortest residence time.

Flow from the outlet began after the start of lake inflow. The daily P & N Lake discharge record for 1980 showed an outflow peak prior to ice-off (Fig. 9), but 66% of the total annual discharge occurred after ice-off (108 d or 80% of the total outflow period), because of several large summer precipitation events. For 1981 the daily discharge record also showed a spring outflow peak before ice-off (Fig. 9), but in contrast with the previous year, 95% of the total discharge occurred during the ice-on period (25 d or 41% of the total outflow period). These and other physical data comparing differences between the 1980 and 1981 water budgets for P & N Lake are presented in Table 1. The initial P & N Lake outflow consisted of cold (<1°C), low-conductivity meltwater, with no distinction between the icemelt (ice compartment) and runoff water (throughflow compartment) as determined from the ^{3}HOH data. A similar occurrence was first noted by Schindler et al. (1974) and has since been shown eleswhere (Henriksen and Wright 1977; Jeffries et al. 1979).

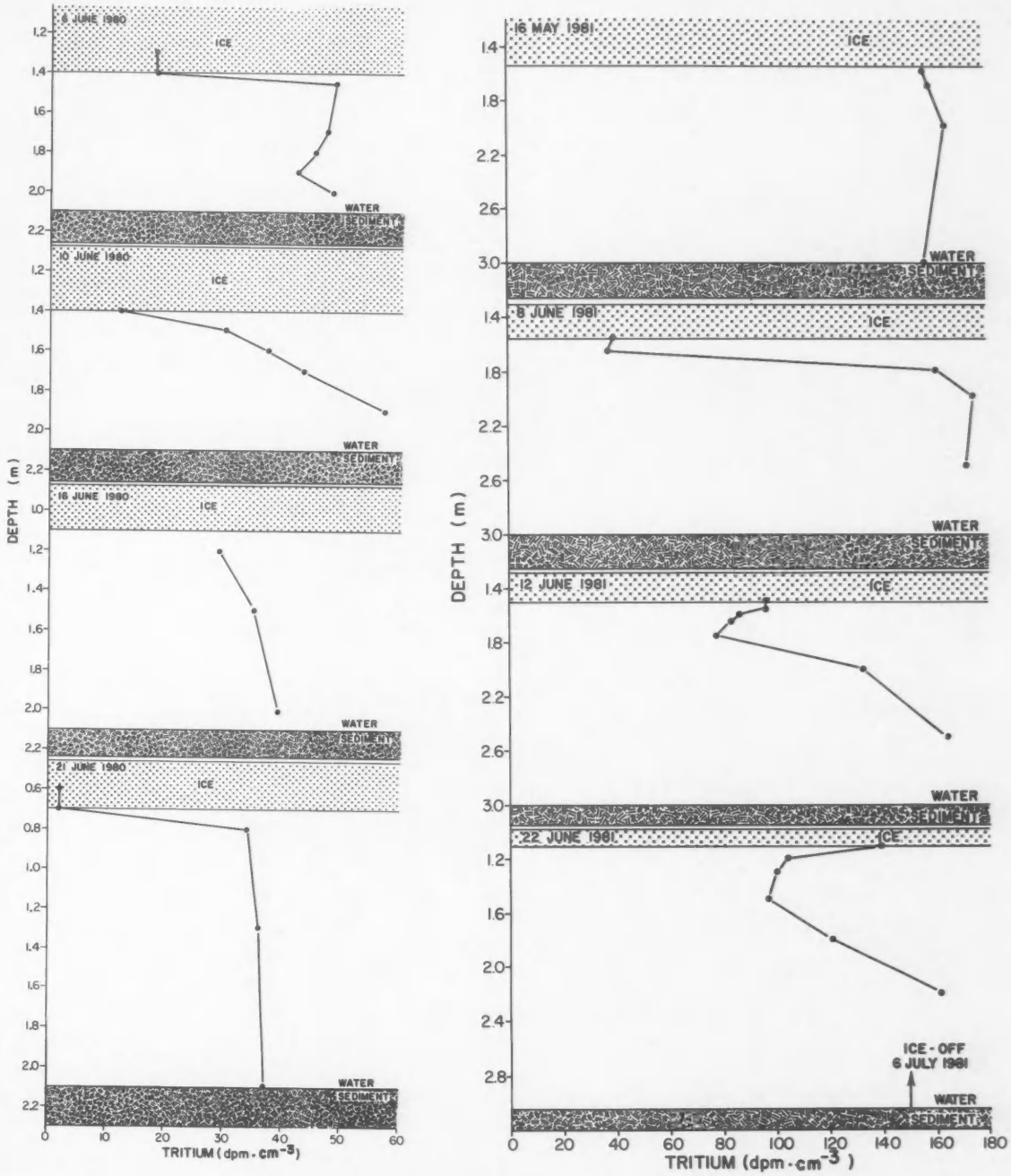

FIG. 3. Profiles of tritium in P & N Lake in 1980 and 1981, 150 m from the outflow, at site A.

Compartment Renewal Times

The theoretical turnover time (TT_c) was predicted from model M1 for each of the three compartments: subice, ice, and throughflow. Table 2 shows the different results determined for the (1) combined hydrological and water budget data (H_2O dataset) and (2) combined tritium (^3HOH) and water budget data (^3HOH dataset). Model M1 predicted that the subice compartment (TT_s) was underestimated and throughflow (TT_i) overestimated for the annual period (first melt to outflow freeze-up). The volume lost from each lake compartment to the outflow was expressed as a percentage of the total outflow loss (Table 3).

Although the two water years 1980 and 1981 were very different, it was necessary to combine the ^3HOH data of both

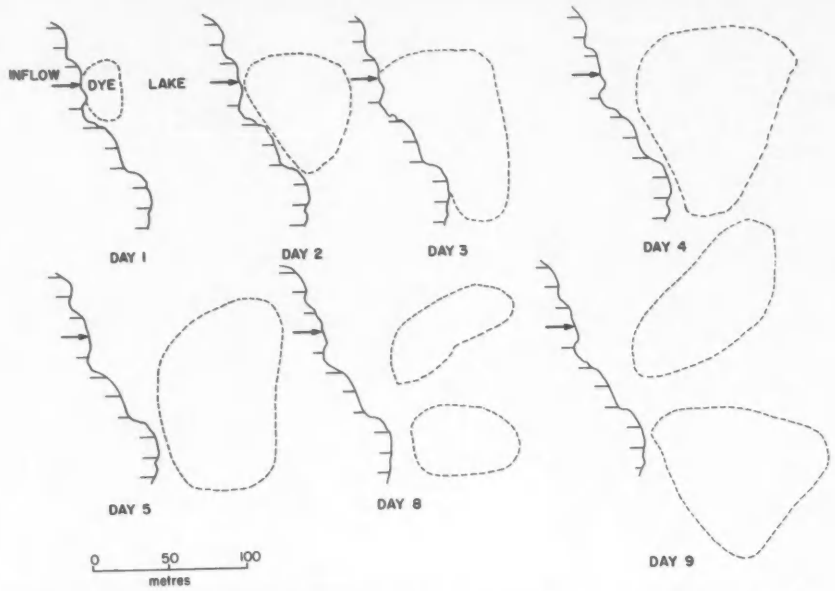

FIG. 4. Plan view of Rhodamine B dye added to an inflow of P & N Lake on 27 May 1980 (day 1).

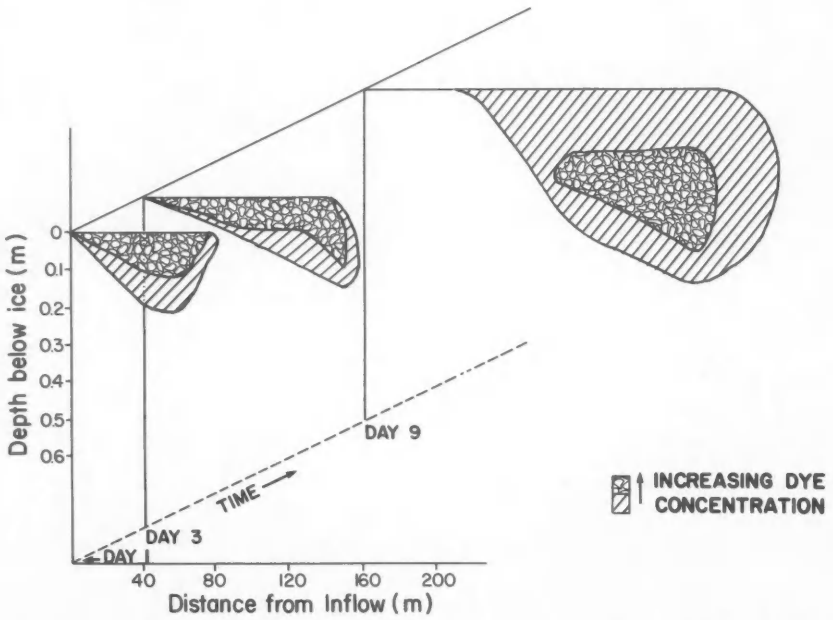

FIG. 5. Vertical distribution of Rhodamine B dye added to an inflow of P & N Lake on 27 May 1980.

years to determine the role of each compartment in the water budget. To do this it was assumed that during any year and within a specific time interval (ice-on, ice-off, annual) the compartment proportions that exit via the outflow remain constant. It was possible to determine these proportions based on the ^{3}HOH and H_2O datasets (model M1). Table 3 indicates that for both time intervals (ice-on and annual period), the estimate of volume lost differed between the ^{3}HOH and water budget data with the greatest deviation occurring during the ice-on period. The theoretical turnover time for each compartment (TT_c, as predicted by model M1), based on the water budget data (H_2O dataset), was higher for the subice and lower for the throughflow than determined from the ^{3}HOH data (Table 2).

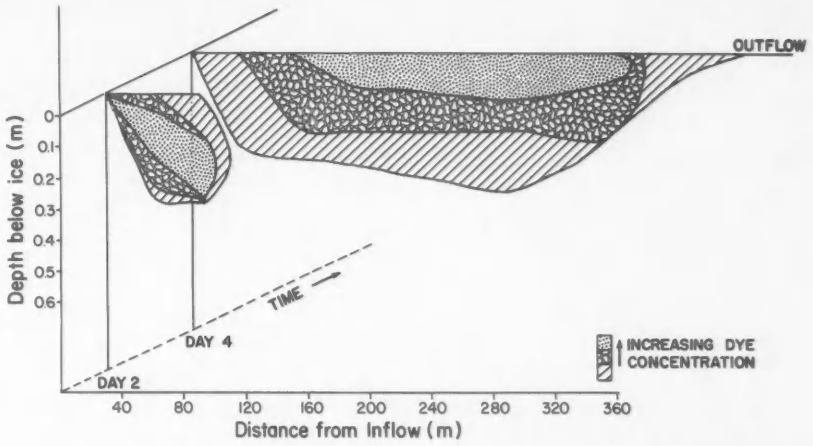

FIG. 6. Distribution of Rhodamine B dye added to an inflow of Spring Lake, 4–7 June 1980.

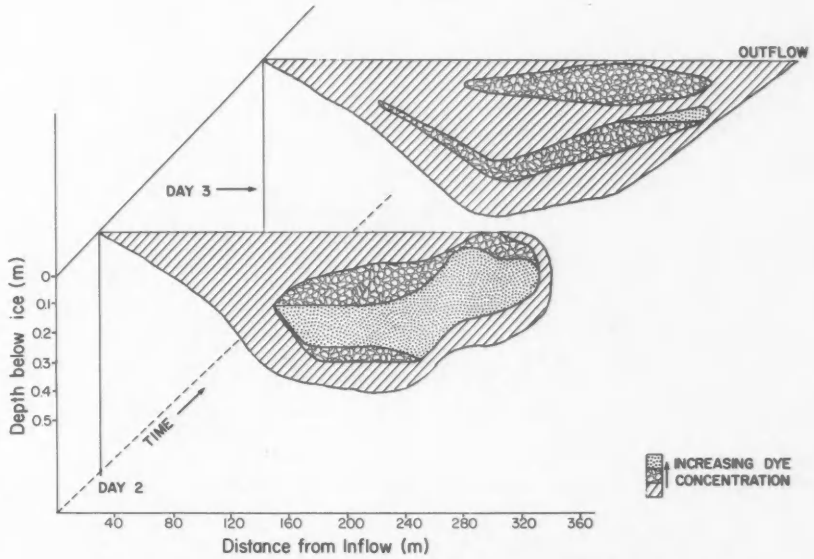

FIG. 7. Distribution of Rhodamine B dye added to an inflow of Hawk Lake, 12–14 June 1981.

To show the chronology of differences between the losses predicted by the traditional complete mixing model and measured losses, estimates of the daily volume lost for the labelled compartment were obtained from the outflow ^{3}HOH data (samples taken at the outflow). Then, these measured data were compared with those predicted from model M2 for the entire ice-on period (Fig. 10). Differences between estimates were greatest at the onset of springmelt and became zero at ice-off.

Discussion

The data we obtained from ^{3}HOH, conductance, and temperature profiles throughout the meltwater period showed a strong stratification beneath the ice which continued to ice-off. These results allow better interpretation of the data from lakes at lower latitudes, where during melt the concentrations of various materials in a water layer immediately beneath the ice are often very different from concentrations in the lake water below. Prentki et al. (1979) reported that during springmelt a layer forms beneath the ice at Lake Wingra, Wisconsin, which is composed of dilute, cold icemelt and runoff from shore. In Lake Wingra (ice thickness approximately 0.3 m), suspended solids, particulate phosphorus, dissolved reactive phosphorus, total nitrogen, and filtered biochemical oxygen demands were higher in the runoff layer (0.5 m) than in the lake water (1.5 m), thus indicating a lack of vertical mixing between compartments. More recently a double inflow spike (^{3}HOH and dye) was carried out at L240 (Experimental Lakes Area, Ontario) which showed the layering of inflowing water beneath the ice (G. Linsey and R. H. Hesslein, Freshwater Institute, Winnipeg, Man., pers. comm.).

The layering of inflowing water beneath the ice has also been

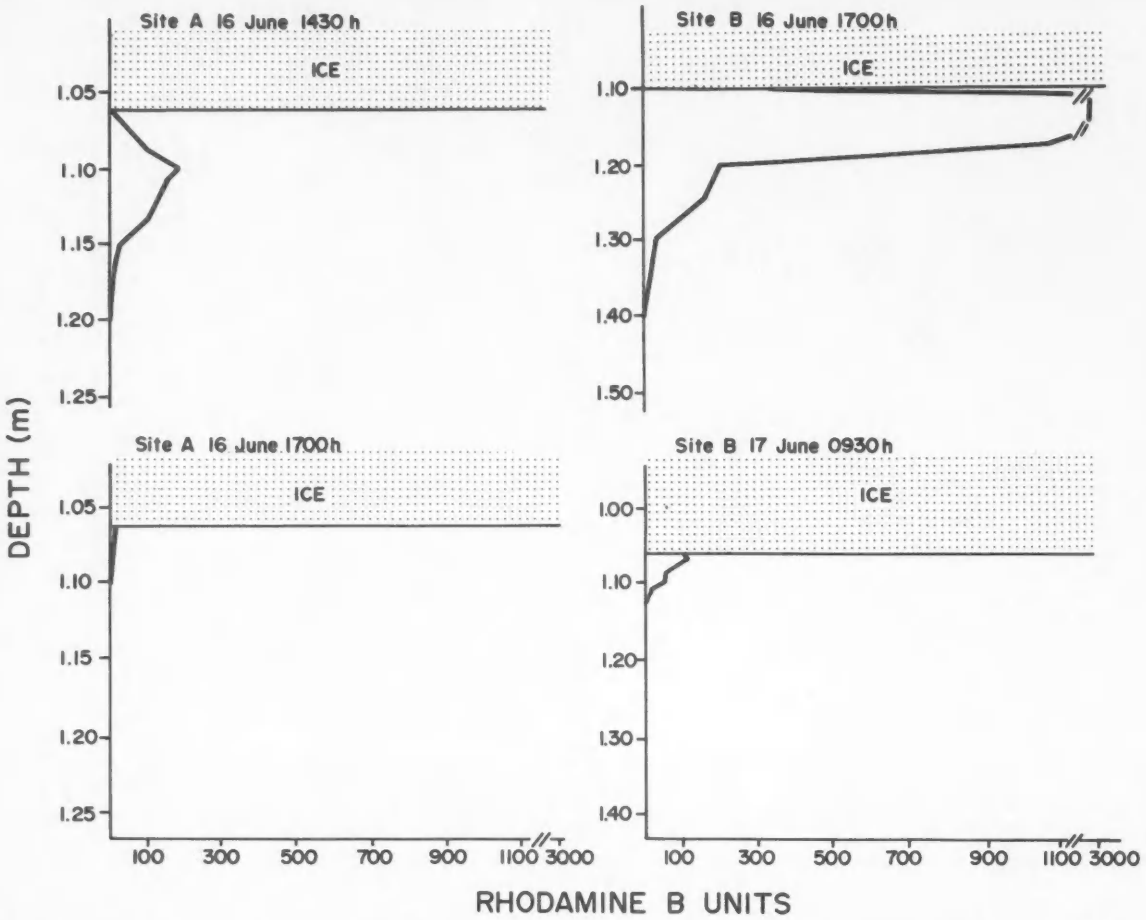

FIG. 8. Profiles of Rhodamine B dye added to the candled ice surface of Turkey Lake at two locations, site A on 16 June and site B on 17 June 1980.

shown by E. Prepas (University of Alberta, Edmonton, Alta., pers. comm.) for Bob Lake (Haliburton, Ont.), where total phosphorus and total dissolved phosphorus were similar at the inflow and directly beneath the ice and higher than the rest of the lake water. Acid precipitation studies on snowmelt in central Ontario (Jeffries et al. 1979), the eastern United States (Hendrey et al. 1980), Sweden (Hultberg 1977), and Norway (Gröterud 1972; Hendriksen and Wright 1977) have shown that during ice-cover the inflowing runoff (which has a low pH) forms a thin layer beneath the ice and is associated with the littoral zone. Trace metal studies in Manitoba (Franzin and McFarlane 1981) also show this layer of snowmelt (direct or runoff) beneath the ice. However, none of these studies have quantified the volumes and flows of the meltwater layers.

Based on the theoretical turnover time for each compartment determined using both the ^3HOH and H_2O datasets, for lakes with no ice-cover and assuming that the lake water is completely mixed and knowing the lake's water budget, the difference between actual and predicted losses (for all compartments) via the outflow should be zero. However, in the arctic the overriding factor that reduces whole-lake mixing is the duration of the ice-cover period. In a year when most precipitation occurs prior to and during melt (but before ice-off) the difference between measured and predicted TT_c will be large, with a large proportion of throughflow and a small proportion of subice lost, as for P & N Lake in 1981. When most of the annual precipitation falls during the ice-off period when the lakes are well mixed, the difference will be smaller.

The greatest proportion of throughflow loss from the lake to the outflow for any year should occur when ice leaves the lake late in the year, when inflows cease early, and when summer precipitation is low. The smallest proportion of throughflow should occur, when the ice-cover period is short, winter snow accumulation is small, and high inflow volumes occur during the open water season. For lakes where ice-cover and/or density differences prevent whole-lake mixing, the actual TT_c for any year will depend on the amount of precipitation, timing of precipitation, duration of the ice-cover, and the volume of the lake basin relative to the size of the tributary drainage area. Deep headwater lakes will show greater differences than shallow lakes with short residence times. We have not attempted to build a more sophisticated general model relating all these factors, partly because a data base suitable for developing and testing such a model is lacking.

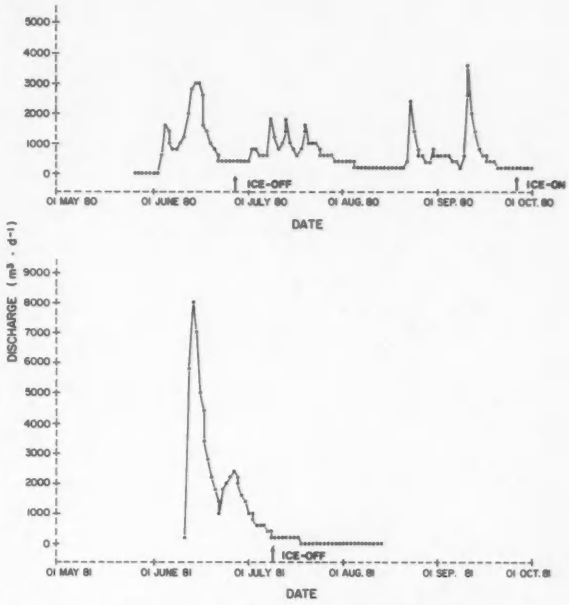

FIG. 9. Outflow discharge from P & N Lake, 1980 and 1981.

TABLE 1. Measured physical data for P & N Lake, 1980 and 1981.

	1980	1981 (to 31 August)
Outflow vol. (m^3)[a]	9.42×10^4	6.70×10^4
Throughflow vol. (m^3)	1.77×10^5	7.10×10^4
Ice vol. (m^3)[b]	8.91×10^4	8.83×10^4
Subice vol. (m^3)[b]	1.35×10^5	1.36×10^5
% annual discharge from first melt to ice-off[a]	36.6 (27 d)	95.0 (25 d)
% annual input from first melt to ice-off[a]	33.7 (27 d)	88.4 (25 d)

[a]1980 (Dalton 1981); 1981 (C. Allan and G. K. McCullough, unpubl. data).
[b]At time of maximum ice depth.

TABLE 2. Turnover times (yr) predicted by M1 for the three P & N Lake compartments for both the annual and ice-on periods.

	Annual period		Ice-on period	
Compartment	^3HOH	H_2O	^3HOH	H_2O
Subice	3.5	2.9	10.1	4.9
Ice	3.4	2.9	4.2	4.9
Throughflow	1.9	2.9	2.4	4.9

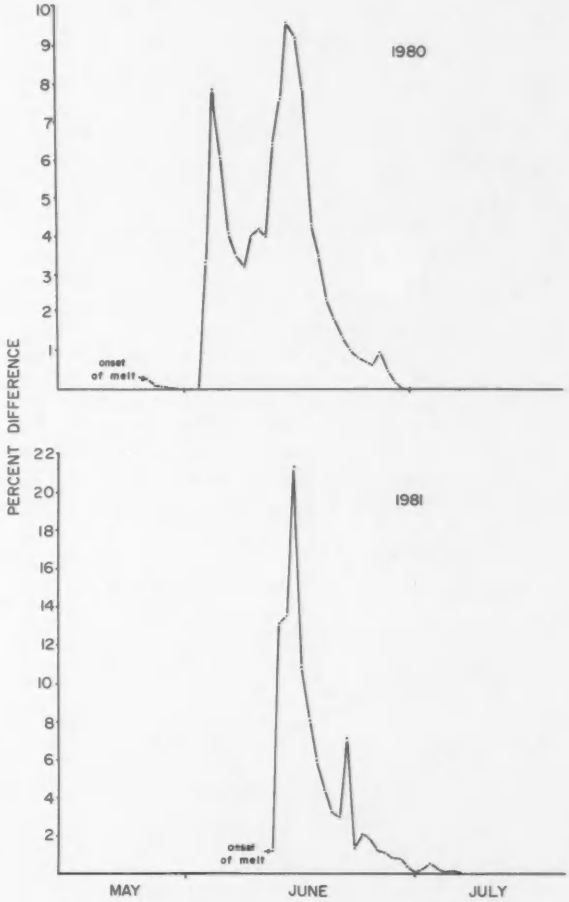

FIG. 10. Daily percent differences between data predicted from model M2 and the measured data for labelled compartment volumes lost in P & N Lake outflow, 1980 and 1981.

TABLE 3. Percent (in parentheses) and volume (m^3) of each compartment of P & N Lake lost to outflow for the combined period 1980–81, based on the H_2O and ^3HOH budget data (M2 model).

Period	Compartment	M2 model	Traditional complete mixing model
Annual	Subice	(35.4) 57 100	(43.1) 69 500
	Ice	(25.9) 47 800	(30.7) 49 500
	Throughflow	(38.7) 62 400	(26.1) 42 100
Ice-on	Subice	(23.0) 21 900	(47.7) 45 500
	Ice	(39.9) 38 000	(33.9) 32 300
	Throughflow	(37.1) 35 300	(18.3) 17 500

Our results show that existing lake loading models, which assume that on an annual basis 100% mixing occurs between the subice, ice, and throughflow compartments prior to loss from the outflow, need reevaluation. For all lakes that have ice-cover for part of their annual cycle, complete mixing of inflowing water probably does not occur.

There are at least three main consequences of the layering of meltwater (snow, ice, and runoff) beneath the ice and its subsequent loss to the outflow. First, Schindler et al. (1974) and H. E. Welch (unpubl. data) have postulated that the cryoconcentrated solutes that accumulate in this subice compartment overwinter remain in the subice compartment and are only

partly lost to the outflow the following spring. Second, the loss of dilute throughflow in the spring, leaving behind cryoconcentrated salts in the bottom water, results in arctic lakes having appreciably higher total dissolved solids than their inflows and outflows (unpubl.). Third, snow, which often contains large amounts of acids and heavy metals, may accumulate on lakes and drainage basins, to be released as meltwater in the spring (Elgmork et al. 1973; Hagen and Langeland 1973; Johannessen and Henriksen 1978; Franzin and McFarlane 1981; Zajac and Grodzinska 1982). During snowmelt, the meltwater that layers beneath the ice is not diluted with the lake water, often resulting in highly polluted waters in the upper lake strata, which may have deleterious effects on the biota (Gröterud 1972; Leivestad and Muniz 1976). Prentki et al. (1979) found that such surface layering of spring meltwater prevented the weedbeds found at a depth of 1–2 m from intercepting the nutrient-rich inflowing water, therefore creating a nutrient-limited environment. In the arctic where the ice-on period may last over much of the year, these effects may be even more pronounced, although research designed to look for these effects in arctic lakes has not been conducted, to our knowledge.

Acknowledgments

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Annulus Development on the Second Dorsal Spine of the Spiny Dogfish (*Squalus acanthias*) and Its Validity for Age Determination

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Beamish, R. J., and G. A. McFarlane. 1985. Annulus development on the second dorsal spine of the spiny dogfish (*Squalus acanthias*) and its validity for age determination. Can. J. Fish. Aquat. Sci. 42: 1799–1805.

Annual marks were visible in three different areas of the dogfish (*Squalus acanthias*) spine. The annuli in the mantle, the stem base, and the inner dentine develop independently of each other. Annuli that formed on the mantle were readily identifiable externally, making them the most useful for age determination. The mantle annulus is an accumulation of pigment that appears to form because enamel deposition is not synchronous with the upward growth of the spine, resulting in the production of darkened bands that often form ridges. We validated our interpretation of annuli from fish aged 20–70 yr by tagging and recovering dogfish that were injected with oxytetracycline. Through validation we were able to demonstrate that some previous studies have underestimated age, resulting in a misunderstanding of important life history parameters.

On a observé des marques annuelles dans trois régions différentes de l'épine de l'aiguillat commun (*Squalus acanthias*). Les anneaux au niveau du manteau, de la racine et de la couche intérieure de dentine se développent indépendamment les uns des autres. Les anneaux qui se sont formés au niveau du manteau étaient facilement identifiables de l'extérieur, ce qui en fait les éléments les plus utiles pour la détermination de l'âge. L'annulus du manteau correspond à un accumulation de pigment qui semble se former du fait que le dépôt d'émail ne se produit pas en même temps la croissance vers le haut de l'épine. Cela entraîne la production de bandes foncées qui forment souvent des crêtes. Nous avons prouvé la justesse de l'interprétation que nous avons faite des anneaux à partir de poissons dont l'âge variait de 20 à 70 ans en étiquetant et en recapturant des aiguillats auxquels on avait injecté de l'oxytétracycline. Nous avons pu ainsi démontrer que l'on avait, dans certaines études antérieures, sous-estimé l'âge, donnant ainsi lieu à une interprétation erronée de paramètres importants du cycle vital.

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Effective management of spiny dogfish (*Squalus acanthias*) off Canada's west coast requires an accurate method of age determination. Dorsal spines have been used by Kaganovskaia (1933), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), Ketchen (1975), and Soldat (1982). While some attempts were made at validation, the method has not been validated using a quantitative technique that is applicable for all age groups (Beamish and McFarlane 1983). In this study we tagged and injected fish with oxytetracycline (OTC) to mark the spines. The recovery of spines from these tagged and injected fish provides a quantitative method of validation. In addition, we studied the development and growth of the spine to determine the method of annulus formation. Markert (1896) and Ritter (1900) described the histology of the spine but did not relate it to spine growth or age determination. Holden and Meadows (1962) have discussed spine growth in relation to age determination but their description of formation of annuli is not in agreement with its histology. Therefore, we reexamined the histology of the spine and the theory of Holden and Meadows (1962) to resolve the question of how an annulus is formed.

Materials and Methods

The second dorsal spine was used in all studies. Spines were removed by cutting horizontally just above the notochord to ensure that the spine base and stem were intact. Spines were cleaned and air-dried in 14.5-kg ungummed Kraft envelopes. Surface annuli were determined using a dissecting microscope. All ages were estimated by a reader experienced in the method outlined by Kaganovskaia (1933), Bonham et al. (1949), Aasen (1961), Holden and Meadows (1962), and Ketchen (1975). The modifications outlined by Ketchen (1975) were used except that all ages were calculated from birth. Most dogfish are born late in the year (Ketchen 1972). The birthdate was assumed to be January 1; however, the age of one was assigned to fish in the second year after birth, when they were 12–14 mo old.

Approximately 400 spines ranging in age from 0 to 4 yr were collected during research cruises in 1978 for measurement of total length (base to tip) and mantle length (measured from ventral anterior base of the mantle to tip). The distance between annuli was measured from a subsample of 20 spines from each of the 2-, 3-, and 4-yr age groups. Cross sections of dried spines

were made by embedding the spine in epoxy and sectioning according to the procedure outlined by Beamish (1979) and Chilton and Beamish (1982).

Longitudinal sections were cut from spines from two fish of age 4 and one each of age 10, 16, and 24. The enamel and pigment were ground away with sandpaper; the exposed surface was then examined according to the procedure of Holden and Meadows (1962).

Fifteen spines collected from younger fish from the Strait of Georgia in 1978 were preserved in Bouins for 48 h and transferred to 70% ethanol and subsequently embedded in paraffin. Histological sections were stained with alizarin red S.

Dogfish from the Strait of Georgia were tagged and given an injection of OTC at the rate of approximately 25 mg/kg body weight (McFarlane and Beamish 1985). The injection procedure was similar to that used for sablefish (*Anoplopoma fimbria*) (Beamish et al. 1983). This dosage was selected because dosages of 75 mg/kg and higher cause mortalities in sablefish (Beamish et al. 1983), and preliminary studies indicated that an acceptable mark was produced at a rate of 25 mg/kg body weight. Spines from injected fish that had been at liberty for 2 yr or more were examined for age validation. Spines from injected fish were processed and examined according to the procedure of Chilton and Beamish (1982). After locating the OTC mark, the distance from the base of the mantle on the anterior edge of the spine to the OTC mark was measured to the nearest 0.1 mm. Under reflected light the position of the mark was located using this measurement and the number of annuli that formed after the mark was counted.

Results

Description of the Spine

The spine is triangular in section and consists of three major structural components; the cartilaginous interior, the stem, and the mantle (Fig. 1). The interior of the spine base is filled with cartilage and surrounded by pulp tissue. The cartilage and pulp tissue degenerate towards the spine tip leaving a central cavity. The cartilage that supports the base of the spine is also continuous with the cartilaginous support for the dorsal fin. The stem surrounds the pulp tissue and is the main body of the spine. The stem consists of three layers of dentine: inner, middle, and outer. The mantle originates at the enamel organ and covers the two anterior–lateral faces of the spine distal to this organ. The midportion of the posterior surface of the spine is not covered by the mantle. The mantle consists of an inner dentine layer, a midportion containing pigment, and an outer layer of enamel.

Cartilage is produced along the ventral and anterior–lateral surfaces of the spine base. The perichondrium (Fig. 1) is visible along the interior of these surfaces. Cartilaginous cells or chondrocytes are distributed within this homogeneous tissue. Towards the tip of the spine the cartilage becomes more cellular and eventually these cells die.

The stem originates close to the ventral surface of the spine base. The anterior–lateral surfaces develop closer to the base than the posterior surface. Odontoblasts at the base of the stem produce an intercellular matrix of collagen fibres and amorphous cementing material. The cementing material is deposited by processes of the odontoblasts into the collagen fibre matrix producing the dentine layer. This layer becomes denser in the centre as it thickens distally. As more dentine is deposited the odontoblasts are displaced progressively further away and the dentinal tubules, formed by the processes of the odontoblasts, become elongated. Dentine is produced on the internal and external surfaces of the stem increasing the thickness and forming an outer, middle, and inner dentine layer. The middle and outer layers are uniform, while the inner layer increases in width from the base to the tip. The anterior–lateral sides of the stem are much thicker than the posterior side. The inner and outer layers are penetrated by dentinal tubules. Upward growth results from the continuous deposition of dentine at the base of the stem, while outward growth is a consequence of cartilage production in the centre of the spine.

A pulp cavity, within the spine, containing nerves and blood vessels is situated between the perichondrium and stem odontoblasts.

The mantle originates at the enamel gland and consists of an outer layer of enamel, a midlayer of pigment, and an inner layer of dentine. Enamel formation is similar to that of dentine in that it involves secretion of an intercellular matrix, followed by calcification of that matrix. The production of pigment and mantle dentine occurs adjacent to the inner surface of the enamel organ. The odontoblasts near the organ produce thin layers of dentine. Pigment (melanin) is produced by the melanophores at the inner surface of the organ and is deposited between the enamel and the dentine. This mantle is laid down on the stem dentine. As this new layer passes the top of the enamel organ, enamel is added from the enamel epithelium (ameloblasts) at the opening of the organ to complete the mantle. As the mantle dentine is laid down, cell-filled canals are formed that make up the marginal canal system, the purpose of which is uncertain although the cells could be responsible for calcification of the dentine.

Darkly pigmented ridges appear on the surface of the mantle. These ridges are used to estimate the age of dogfish. While the method of age determination has been described as counting darkened bands (Holden and Meadows 1962; Ketchen 1975), we define the annulus to be a darkened band or ridge or both. In most cases the ridges are darkened; however, occasionally a ridge does form that is not darkened.

When smoothing the ridges from five spines using sandpaper, we observed that the ridge could be removed from all spines without disturbing the pigment layer. The pigment layer could be removed without substantially disturbing the dentine layer. This indicates that the ridges consisted of thickened enamel.

We believe the annulus forms because the mantle formation is not synchronous with the upward growth of the spine. When spine growth is reduced there is a concentration of pigment and a thickening of the enamel.

Spine Lengths

There was no significant difference between the mean lengths of similar aged fresh and dried spines (Table 1). There also was no difference in the mean lengths of similar aged spines from males and females. Thus, all measurements were made using dried spines and combining males and females. The average growth of the spine during the first, second, third, and fourth years was 0.33, 0.16, 0.04, and 0.25 cm, respectively (Table 1). The length of the nonenameled portion of the stem (total length minus mantle length) ranged from 0.81 to 1.09 cm for this same period (Table 1) or 0.07 cm/yr. Thus, the total spine growth in the first 4 yr was not sufficient to cause dentine formed at the base of the stem to be covered with enamel.

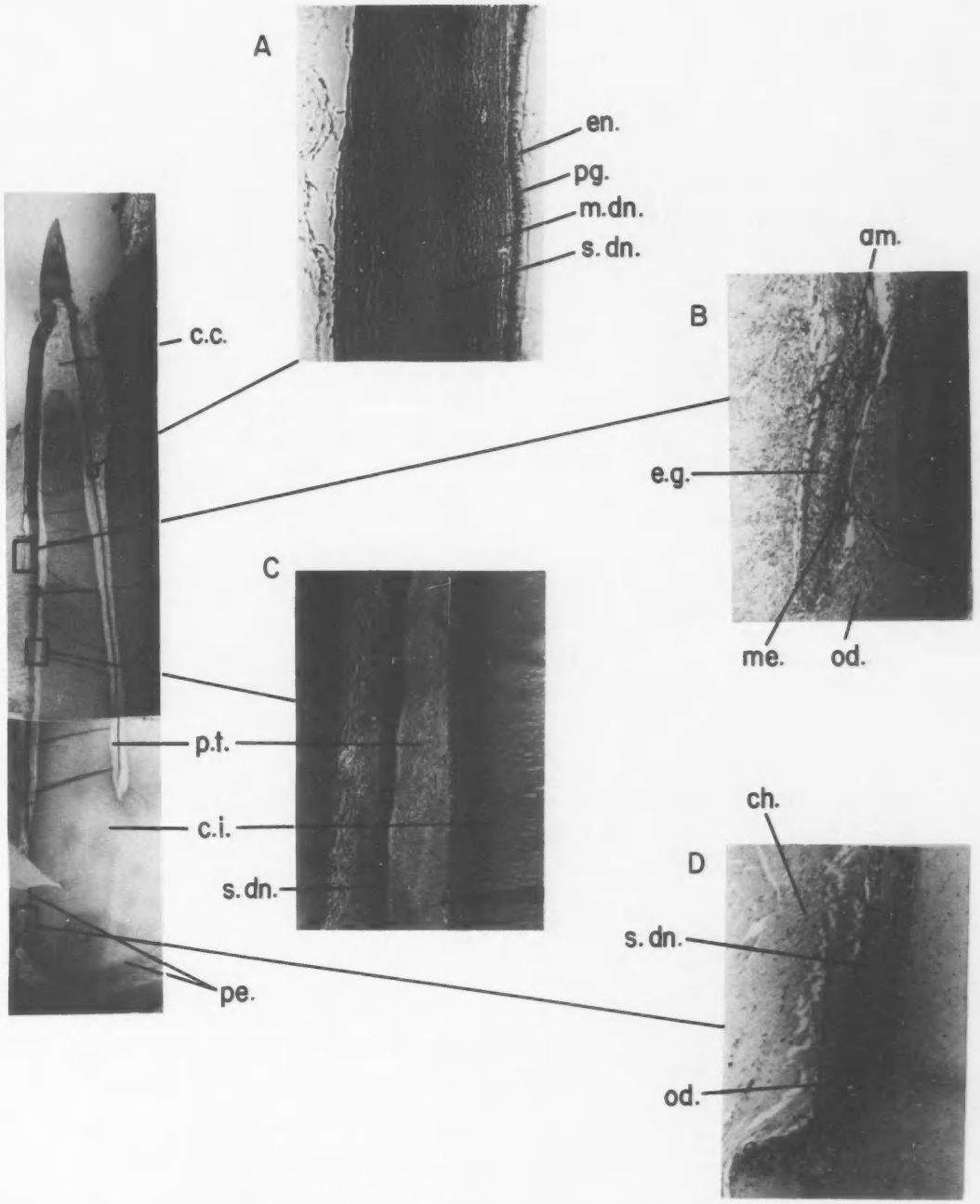

FIG. 1. Longitudinal section through a dogfish spine (extreme left): c.c. = central cavity, p.t. = pulp tissue; c.i. = cartilaginous interior; pe. = perichondrium. (A) Posterior edge: en. = enamel; pg. = pigment; m.dn. = mantle dentine; s.dn. = stem dentine. (B) Enamel gland: e.g. = enamel gland; od. = odontoblasts; me. = melanophores; am. = ameloblasts. (C) Anterior edge of stem. (D) Anterior laterial area of stem formation: ch. = chondrocytes.

TABLE 1. Measurement of fresh and dried dogfish spines for fish aged 0+ to 4+.

Age	No. of observations	Total spine length (cm)	Mantle length (cm)	Stem base (nonenamelled portion)
		Dogfish spine lengths, fresh		
0	3	2.02±0.05	1.21±0.04	0.81±0.01
1	9	2.41±0.21	1.47±0.17	0.94±0.07
2	19	2.64±0.17	1.62±0.12	1.02±0.07
3	30	2.68±0.14	1.64±0.11	1.04±0.06
4	18	2.78±0.20	1.69±0.16	1.09±0.11
Mean annual increment		0.19±0.16	0.12±0.11	0.08±0.06
		Dogfish spine lengths, dried		
0	29	2.05±0.30	1.25±0.24	0.81±0.10
1	47	2.38±0.18	1.49±0.11	0.89±0.10
2	63	2.54±0.20	1.59±0.10	0.96±0.16
3	69	2.59±0.19	1.62±0.14	0.98±0.09
4	58	2.84±0.20	1.77±0.15	1.08±0.09
Mean annual increment		0.20±0.12	0.13±0.09	0.07±0.03

Growth Pattern in the Stem

When the cleaned and dried stem (spine) base is viewed with reflected light there is a pattern of alternating growth zones. When the mantle is scraped off, it is possible to see that this pattern extends beneath the mantle towards the spine tip. These zones are the "cone bases" described by Holden and Meadows (1962). The shape of the growth zones is identical to the shape of the spine base, suggesting that they form as a consequence of growth changes at the spine base. Injected fish also had an OTC mark at the spine base (Fig. 2A). The number of zones that formed ventral to this mark on the 20 specimens examined was equal to growth periods at liberty, indicating that these marks were formed annually. However, these stem annuli had a different shape than the mantle annuli and clearly were not formed in the same manner.

Cross sections from 12 spines revealed growth checks only in the inner dentine. They were difficult to count, being separated from each other by a number of less obvious rings. At the outer edges these checks become closely spaced, irregular, and difficult to define, particularly in fish older than 10 yr. The outer dentine shows only a few obscure rings. Cross sections of five spines from fish injected with OTC did have an OTC mark in the inner dentine, indicating that at this time, growth in the inner dentine appears synchronous with growth at the spine base and the mantle.

Validation

As of December 31, 1983, 13 826 fish were injected with OTC and released. A total of 369 fish have been recaptured of which the spine was recovered from 261 fish. Sixty-six percent had an OTC mark. For this validation study we used fish that had been recovered after being at liberty for 2 yr or more. Fish that were at liberty less than 2 yr were excluded because the slow growth of the spine made it difficult to separate the OTC mark from the annulus. Included in this report is one fish recaptured in 1984 after 4 yr at liberty and one fish recaptured in January 1985 after 5 yr at liberty (Fig. 3A, 3B).

There were 11 fish that had been at liberty for 2 yr, five fish for 3 yr, one fish for 4 yr, and one fish for 5 yr. Those fish that had been at liberty for 2 yr had developed two annuli below the mark, and four fish at liberty 3 yr had three annuli below the mark (one fish had two annuli below the mark), and the single individuals recovered after 4 and 5 yr had developed four and five annuli, respectively (Table 2). In only one case was there a disagreement with the years at liberty (growth periods) and the number of annuli.

There was a bias in all readings resulting from a knowledge that injected fish could have only been at liberty for a few years. However, in these samples the identification of annuli was not confused by checks or false annuli and was relatively uncomplicated.

To test this bias, all annuli on the mantle of the spine were counted. The spine was reexamined using UV light and the number of annuli that formed prior to tagging and injection (distal to OTC mark) were counted. The years at liberty were then determined by subtracting this latter estimate from the former. In all cases the years at liberty were estimated correctly.

In most of the samples the annulus was easily identified as a ridge that was darker than the areas above and below it. In some older fish, the annuli that formed after the OTC mark were quite closely spaced (Fig. 2B). Under reflected light the annuli were very distinct, forming prominent darkened ridges. In younger fish the annuli tended to be more widely spaced and ridges were less prominent.

Fish that had been at liberty for approximately 2 yr or more ranged in age from 20 to 70 yr (Table 2) and in length from 61 to 111 cm. The tip of the spine showed some wear after an average age of 8–10 yr. Ages older than this were estimated using the relationship between the width, the no-wear point, and spine growth (Ketchen 1975).

The OTC mark frequently appears as a double line (Fig. 2B, 3A, 3B). Since the spine growth is slow and OTC remains active for only a short period of time (Milch et al. 1958), the two lines must have developed simultaneously. The formation of two lines confirms that mantle dentine forms first and subsequently is covered with enamel. This means that there are distinct and separate sites for mantle dentine and enamel formation.

Discussion

Problems associated with not validating age determinations have been discussed by Beamish and McFarlane (1983). In the absence of a properly validated method for aging dogfish, investigations have grouped growth zones on the spine (Holden and Meadows 1962), or rejected difficult to age spines (Bonham et al. 1949; Holden and Meadows 1962; Ketchen 1975). Unfortunately, in the absence of validation there is no scientific basis for ignoring, grouping, or eliminating material where growth zones are visible. Where this has been done (Bonham et al. 1949; Ketchen 1975), it appears that younger ages may have been produced or there has been a selection for faster growing fish which frequently lay down clearer annuli.

Several studies have attempted to validate this method. While Holden and Meadows (1962) stated that the bands they counted were "proved to be annuli," they presented no substantiation. Ketchen (1975) used a variety of techniques, including length frequency analysis and mercury accumulation. Again, these techniques cannot validate an age determination method over the age range of the species. In addition, we have examined length frequencies from a number of locations, and because of

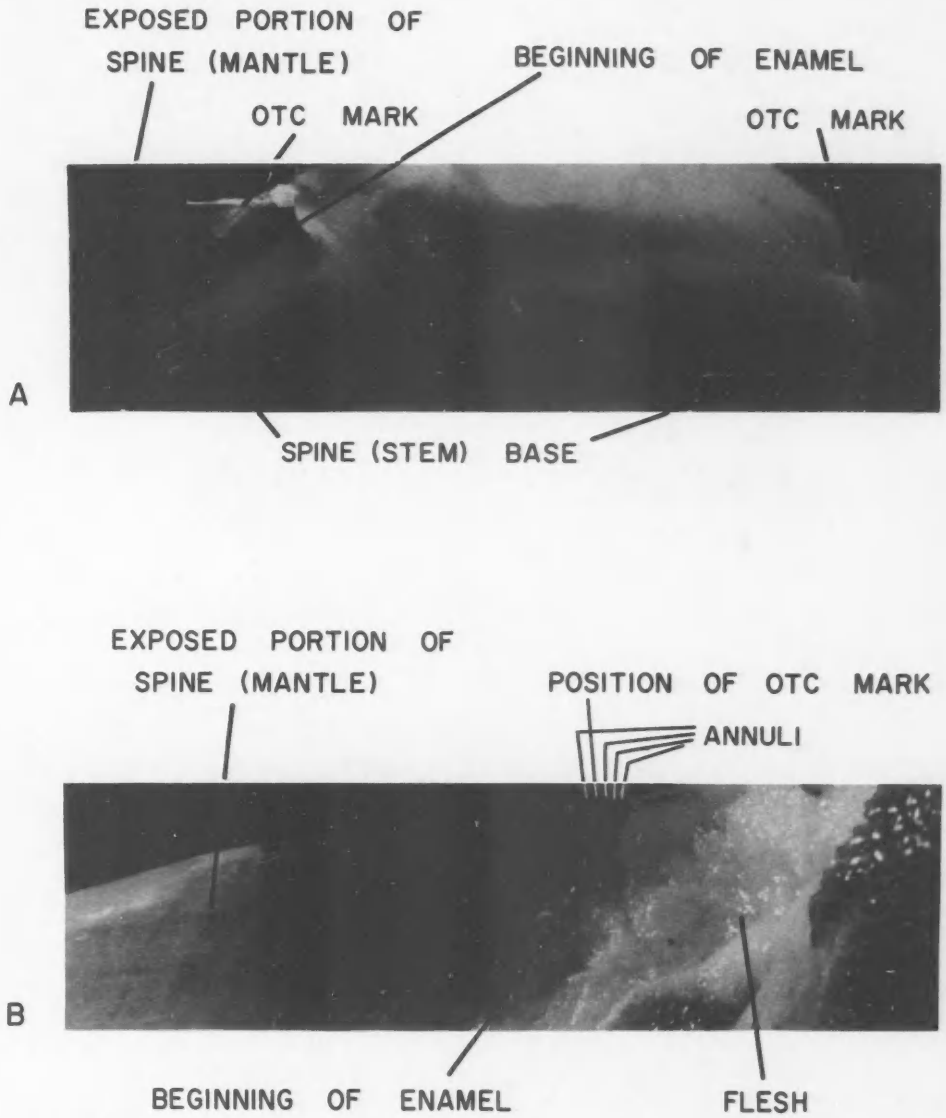

FIG. 2. Dorsal spines from recaptured tagged and injected dogfish. (A) Spine from a 75-cm male recaptured in October 1983 after 2 yr at liberty showing that the OTC mark forms both in the mantle (double line) and the stem dentine. (B) Spine from a 111-cm female recaptured in November 1983 after 3 yr at liberty showing slow growth as indicated by the spacing between annuli.

the slow growth rate it is difficult to identify age groups (Beamish et al. 1982). Jones and Geen (1977) used X-ray spectrometry to show that concentrations of calcium and phosphorus in vertebrae corresponded to the annulus. However, this does not prove that the growth zone or the concentration of the minerals occurs annually.

In our study the placement of a "OTC mark" and subsequent recapture of individuals does allow examination of the growth zones that form during the period at liberty. Because the annulus did develop once during the year for fish aged 20–70 yr, we have demonstrated that the growth zones on the second dorsal spine can be used for age determination. We concluded that all darkened bands, ridges, or both must be counted. In most cases the ridge was also darkened. In addition an annulus could be identified on the stem. This annulus was visible on dried specimens in the base of the spine and was visible when the mantle was removed. However, because of the difficulty in removing the mantle, we recommend that growth zones on the mantle be used for age determination.

Our description of the histology of the spine was identical to the descriptions of Markert (1896) and Ritter (1900). However, our description of the growth of the spine and annulus formation does not agree with those of Holden and Meadows (1962) and Soldat (1982). Holden and Meadows (1962) stated that the stem

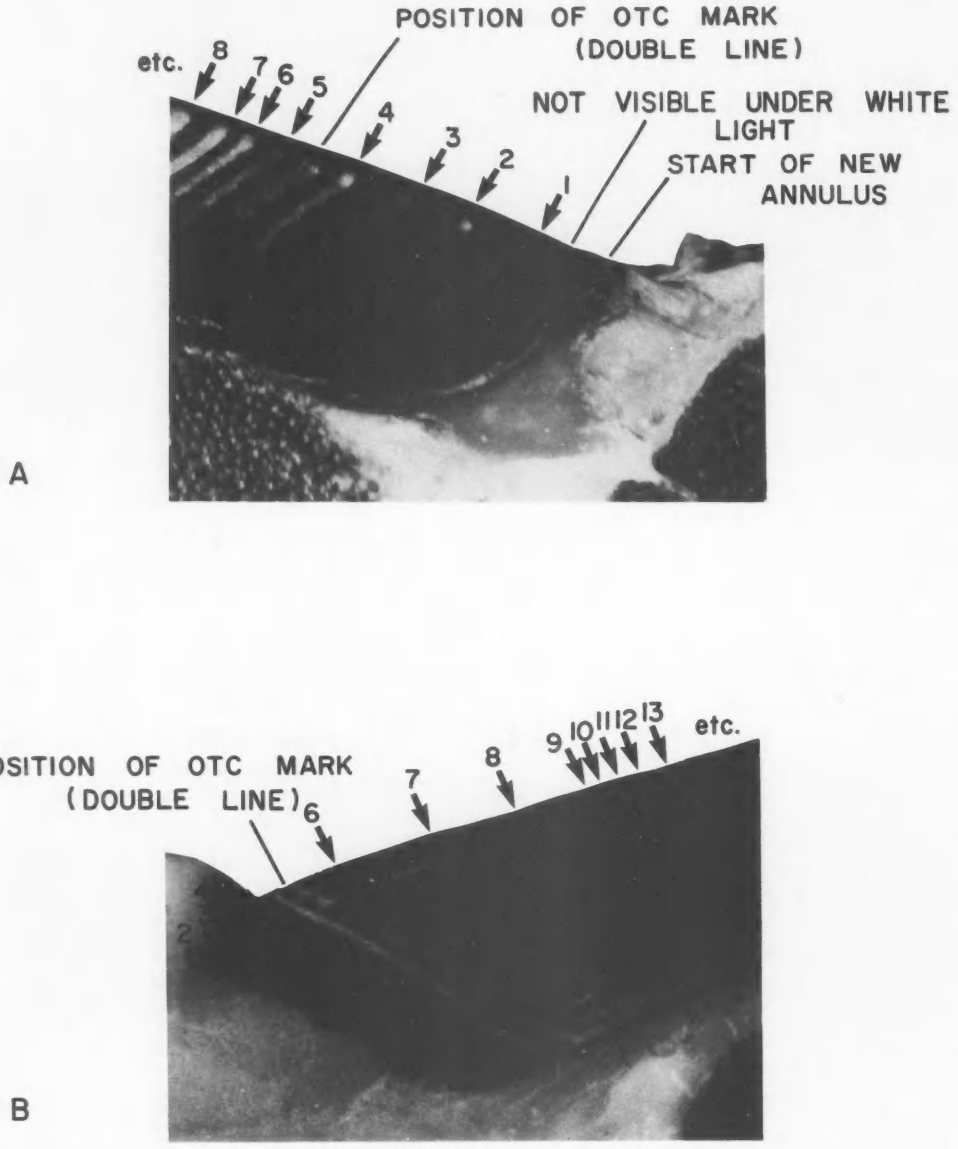

FIG. 3. (A) Spine from a 75-cm male recaptured in late 1984 after 4 yr at liberty. Photographed using UV light. (B) Spine from a 91-cm female recaptured in January 1985 after 5 yr at liberty showing variable spacing between annuli. The annuli that formed after the fish was injected are closely spaced and could be incorrectly grouped. Photographed using UV light.

annulus was "reflected in the external surface by bands of pigment associated with ridges of enamel." They assumed that the spine grew in a series of overlapping cones with the point of overlap being the ridge.

Our study clearly showed that the mantle annulus was formed independently of the stem annulus. Stem annuli result from differential growth at the base of the spine and slowly move away from the base. A number of these annuli may be incorporated in both the unexposed and exposed portions of the spine. Beneath the mantle these growth zones are different in width and shape than mantle growth zones, and chronologically are younger. Since the pattern is produced at the base of the spine, there are no overlapping cones, but rather it is a discontinuous growth pattern with the stem.

The mantle annuli form as a result of a lack of synchrony between the upward growth of the stem and the mantle formation. During periods of slow upward growth it appears that mantle growth is not correspondingly reduced, resulting in a buildup of enamel and a concentration of pigment producing a darkened ridge.

Holden and Meadows (1962) showed that alternating growth zones could be observed in cross sections of the inner dentine.

TABLE 2. Estimated number of annuli that formed on spines from fish tagged and injected with OTC that had been at liberty for 2 yr or more.

Expected no. of annuli (yr) beyond OTC mark[a]	*n*	Estimated no. of annuli	Age (yr) at time of recapture[b]
2	11	2, 2, 2, 2, 2, 2, 2, 2, 2, 2, 2	20, 26, 29, 30, 31, 32, 37, 39, 47, 52
3	5	3, 3, 3, 3, 2	26, 27, 70
4	1	4	26
5	1	5	40

[a]Annulus formation was assumed to occur from November to April. A fish that was expected to form two or more annuli had to be at liberty for at least two consecutive November–April periods.

[b]Ages could not be estimated on all spines because portions were damaged.

They felt that this alternating pattern of growth occurred annually. The maximum number of annuli, in cross section, occurred just dorsal to the apex of the pulp cavity. We also observed this pattern of alternating growth zones in the inner dentine, but they were not as prominent as the mantle annuli.

The increase in thickness of the inner dentine as it approaches the apex of the spine indicates that the perichrondrial layer adjacent to the pulp cavity continues to deposit dentine along the internal face of the stem. Spine growth and spine wear eventually result in a loss of annuli. While this is a third process by which annuli can be identified in the spine, this loss of annuli and the difficulty of their interpretation makes this aging method unsuitable.

Annuli were found in the cross sections of the inner dentine, longitudinally in the stem, and as darkened ridges in the mantle. Sections of the inner dentine were unsuitable as previously described. The annuli visible longitudinally in the stem could be used for age determination but it was difficult and time consuming to remove the mantle. Therefore, we conclude that the mantle annuli were the easiest to identify. The procedure of measuring the thickness of the "no-wear point" (Ketchen 1975) appears to be the only method of estimating lost annuli. While this measurement probably introduces an important error, we were unable to find a better method of estimating lost annuli.

Our validation of mantle annuli indicated that there is no basis for the "grouping" of darkened ridges (Fig. 2B, 3B). This means that ages assigned by others may underestimate the true age. Our estimates indicate that dogfish are older, later maturing, and slower growing than previously thought.

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Mike Smith participated in all aspects of this study, which benefitted from his long experience with dogfish. A number of fishermen and staff of the Fisheries Research Branch participated in the tagging program. Students and staff of the Campbell River and Southgate Secondary Schools also assisted in the study and we appreciate the continued cooperation of V. Egan and D. Brown. Histological sections were prepared by John Bagshaw.

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Optimizing Sampling Design for Herring Spawn Surveys in the Strait of Georgia, B.C.

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Three estimators for two-stage sampling designs assuming unequal sized primaries (transects) were compared. The ratio estimator was found to provide the most consistent estimates of the mean and variance and so was used for estimating optimal sample design. Preliminary results from some biased sampling during 1976 and 1978 provided guidelines for the 1981 study designed to derive an optimal sampling design. Inconsistent results from the two areas surveyed during 1981 prevented general conclusions, but a corroborating resurvey of one area in 1983 suggested that a sampling intensity of five samples per 100 m of transect and transects every 250–400 m along the length of the spawn should result in estimates of the mean egg density with a standard error no greater than 25%. Systematic sampling is logistically preferable to random sampling and can be incorporated into the two-stage design described herein which should be used in future spawn surveys designed to estimate spawning escapement.

On compare trois estimateurs pour des plans d'échantillonnage à deux volets en admettant des transects de taille inégale. Comme l'estimateur du rapport fournissait les estimations les plus uniformes de la moyenne et de la variance, il a été utilisé pour l'estimation du plan optimal d'échantillonnage. Des résultats préliminaires recueillis au cours de certains échantillonnages faussés menés en 1976 et 1978 ont fourni des indications pour l'étude réalisée en 1981 visant l'élaboration d'un plan optimal d'échantillonnage. Des résultats incompatibles recueillis en 1981 dans les deux zones expérimentales n'ont pas permis la formulation de conclusions générales; par contre, un nouveau relevé corroboratif d'une des zones réalisé en 1983 porte à croire qu'une intensité d'échantillonnage de cinq échantillons par 100 m de transect et que des transects situés à tous les 250 à 400 m le long des frayères devraient fournir des estimations de la densité moyenne de la rogue avec une erreur-type ne dépassant pas 25 %. Au point de vue logistique, l'échantillonnage systématique est préférable à l'échantillonnage aléatoire et peut être incorporé dans le plan à deux volets décrit. À l'avenir, celui-ci devrait être utilisé pour les relevés de la rogue conçus pour évaluer l'échappée.

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The foundation of any comprehensive fisheries management regime is an accurate assessment of population abundance. Pacific herring (*Clupea harengus pallasi*) are unique in that they spawn intertidally and concomitantly release large quantities of milt which discolours the water and thus provides a temporarily visible record of the general distribution of eggs. Consequently, surveys of this spawn deposition provide a means for accurately and precisely estimating the size of the population escapement given data on fecundity and age composition. Combined with total catch this provides a very accurate assessment of the prefishery spawning biomass in any geographical area for management purposes. It is the objective of this report to provide guidelines on the sampling intensity required to obtain acceptable estimates of precision for the mean egg density estimates in any individual spawning locality.

Hart and Tester (1934) first proposed spawn enumeration as a means for estimating population abundance. Canadian Fisheries and Oceans personnel have routinely estimated the length, width, and intensity of herring spawns along most of the British Columbia coast since 1950 to provide an index of abundance for fishery management. These surveys are largely shore based and concentrate on the intertidal zone.

In the mid-1970s, research was initiated into the use of SCUBA divers to directly census both intertidal and subtidal herring spawn to obtain more accurate estimates of stock abundance (Humphreys and Haegele 1976). There are two components to this procedure: an estimate of the total area covered by spawn and an estimate of the intensity of spawn or the density of eggs per square metre. The latter may be obtained directly through the labour intensive counting of eggs or indirectly through predictive models (Haegele et al. 1979a; Schweigert and Fournier 1982). In either instance an important consideration is the number of samples required to obtain acceptable estimates of precision of the mean egg density (standard error of 25%) for the entire area of egg deposition.

Sampling always incurs certain costs which in most biological situations are not negligible. The objective of optimizing sampling design is thus either to minimize the variance for a fixed total cost or to minimize cost for a fixed variance (Cochran 1977, p. 280). This paper presents the results of a 1981 study designed to derive the minimum cost design for a fixed

predefined standard error of the mean. We compare the relative efficiency of various two-stage sampling designs for herring spawn surveys and therefrom derive an optimal sampling scheme which should be employed in subsequent surveys designed to estimate spawning escapement.

Materials and Methods

The sampling procedure for spawn surveys that has evolved through trial and error since 1975 is to set transects of light gillnet leadline perpendicularly from shore seaward (Haegele and Humphreys 1977; Haegele et al. 1979b). Specified quadrats are then sampled along each transect. Transect locations and sample quadrats for each transect were generated from a random number table for the 1981 study. In the other three years, sampling was done in a systematic fashion with transects being evenly spaced at varying intervals along the beach and quadrat samples collected every 20 m along the transect.

The sampling procedure is described in detail in Schweigert and Fournier (1982). We used 0.50-m^2 quadrats in the 1981 study to obtain more representative samples of the large foliose red and brown algae that often eluded the 0.25-m^2 quadrats used in earlier studies. In 1983 we used 0.25-, 0.50-, and 1.00-m^2 quadrats on different transects to examine the effects of quadrat size on the density estimates. The samples, after collection, were separated into vegetation classes and a subsample sufficient to fill a 1-L jar was preserved in Gilson's fluid for subsequent egg enumeration. After digesting away the plant material, two weighed subsamples were counted and if they agreed within 5%, the mean value was taken as the estimate of egg density for that vegetation fraction. Egg estimates for each fraction within a sample were summed to obtain the estimate for that quadrat.

Analysis of information collected by the systematic subsampling procedure at Nanoose Bay and Icarus Point in 1976 (Haegele and Humphreys 1977) and in the French Creek and Qualicum areas during 1978 (Haegele et al. 1979b) using a two-stage sampling design indicated that 2–6 samples per 100 m of spawn width and 2–16 transects·km^{-1} of spawn length should result in a standard error of 25% of the mean (see Table 3).

The study area chosen for this work was Lambert Channel and the east coast of Denman Island (Fig. 1). To evaluate the results from the 1976 and 1978 surveys we collected 4 samples per 100 m of transect with 10 transects·km^{-1} at Fillongley Park and 3 samples per 100 m of transect with 5 transects·km^{-1} at Longbeak Point in 1981 (Fig. 2). As part of the survey to estimate spawning escapement for this area we resampled Fillongley Park using 5 samples per 100 m of transect and 10 transects·km^{-1} in 1983. Similarly, at Whalebone Point we collected 5 samples per 100 m of transect but only sampled 4 transects·km^{-1} (Fig. 3). The 1983 survey was not designed to investigate optimal survey design but nevertheless provided data that could be used to corroborate earlier results.

The sampling procedure we have found to be most suitable from the logistic and practical standpoint falls naturally in the realm of two-stage sampling designs. To compare various sampling designs one can calculate their efficiency relative (RE) to one another or to a standard such as simple random sampling (SRS) by determining the ratio of the variances for the designs to be evaluated. For SRS estimates of the population, mean and variance are obtained from the sample by

$$(1)\quad \bar{y}_{SRS} = \sum_{i=1}^{n} \frac{y_i}{n}$$

$$(2)\quad v(\bar{y}_{SRS}) = \left(\frac{N-n}{N}\right) \sum_{i=1}^{n} \frac{(y_i - \bar{y}_{SRS})^2}{n(n-1)}$$

where y_i = egg density for sample i, n = total number of samples collected, and N = total number of quadrats in the population. We determined the means and variances for three two-stage designs assuming unequal primary unit (transect) sizes (Jessen 1978). The simplest estimator requires no extra sample information but it is biased and will provide particularly misleading results if the spawn width, M_i, and the mean egg density $\bar{y}_i$, for the transect are correlated. The sample mean in this instance is given by

$$(3)\quad \bar{\bar{y}}_1 = \sum_{i=1}^{n} \frac{\bar{y}_i}{n} = \text{mean egg density for the spawn}$$

$$(4)\quad \bar{y}_i = \sum_{j=1}^{m} \frac{y_{ij}}{m_i} = \text{mean egg density per transect } i$$

where y_{ij} = egg density per square metre for sample j in transect i, m_i = number of samples in transect i, and n = number of transects per spawn. The variance estimator for the sample is very similar to that for equal primary units and is given by

$$(5)\quad v(\bar{\bar{y}}_1) = \frac{(1-f_1)s_1^2}{n} + \frac{f_1(1-f_2)s_2^2}{\bar{m}n}$$

where

$$(6)\quad s_1^2 = \sum_{i=1}^{n} \frac{(\bar{y}_i - \bar{\bar{y}}_1)^2}{n-1} = \text{variance among transect means}$$

$$(7)\quad s_2^2 = \sum_{i=1}^{n} \sum_{j=1}^{m} \frac{(y_{ij} - \bar{y}_i)^2}{n(m_i - 1)} = \text{variance among samples within a transect}$$

$$f_1 = \frac{n}{N} = \text{proportion of transects surveyed}$$

$$f_2 = \bar{m}/\bar{M} = \text{proportion of quadrats sampled in transect } i$$

where $\bar{m}$ = mean number of quadrats sampled per transect and $\bar{M}$ = universe estimate of average spawn width. In this case we use total area of spawn divided by total length of spawn for each location.

The other two estimators require extra sample information. They require that the transect length or estimated spawn width, M_i, as well as the universe estimate of average spawn width, $\bar{M}$, be known. The second ($\bar{\bar{y}}_2$) is unbiased and is weighted by primary unit size (transect length), while the third ($\bar{\bar{y}}_3$) is the ratio of the estimated totals in the sample primaries to their total sizes. It has bias which becomes negligible with large n (Cochran 1977).

The formulae for these two means are

$$(8)\quad \bar{\bar{y}}_2 = \frac{1}{n\bar{M}} \sum_{i=1}^{n} M_i \bar{y}_i$$

$$(9)\quad \bar{\bar{y}}_3 = \sum_{i=1}^{n} \frac{M_i \bar{y}_i}{\sum_{i=1}^{n} M_i}$$

where M_i = the number of possible quadrats in transect i, i.e. the transect length. The recommended sampling procedure for unequal sized primaries is proportional allocation which makes

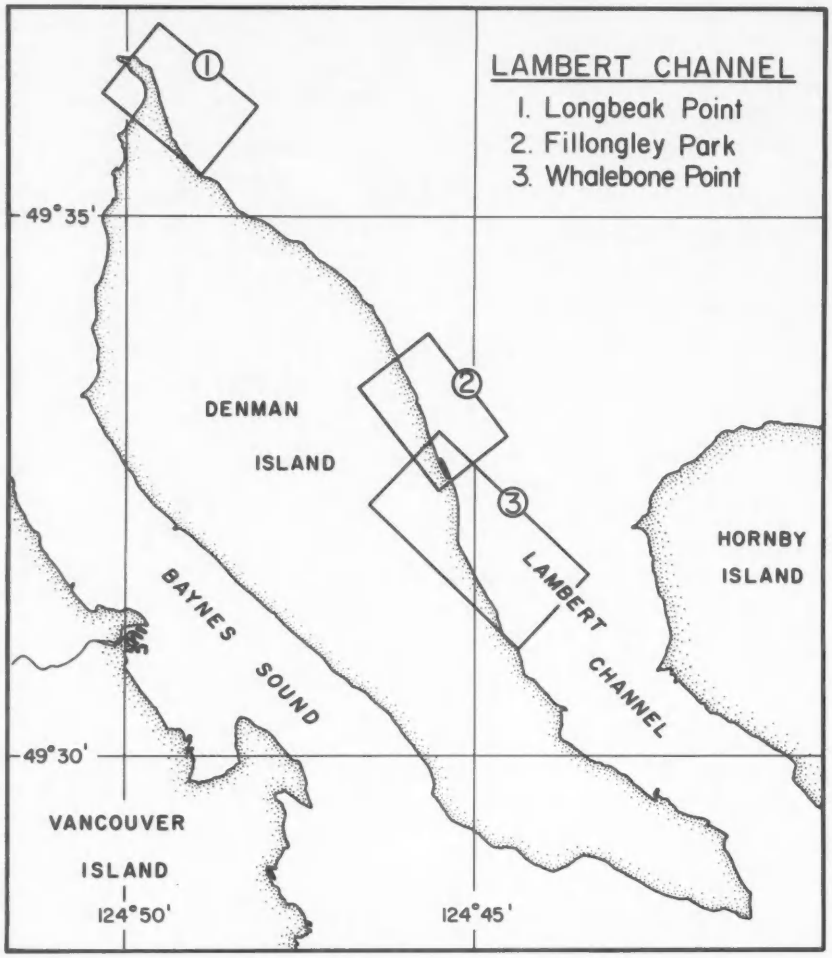

FIG. 1. Area surveyed during the Lambert Channel spawn surveys during March 1981 and 1983.

the variance estimators self-weighting and simplifies the calculations. Jessen (1978, p. 293) presented the appropriate formulae which are identical to those of Cochran (1963, p. 304 and 300, respectively). The self-weighting estimators for the sample are

$$(10)\quad v(\bar{\bar{y}}_2) = \frac{(1-f_1)}{n}\sum_{i=1}^{n}\frac{((M_i/\bar{M})\bar{y}_i - \bar{\bar{y}}_2)^2}{(n-1)} + \frac{f_1}{n}\sum_{i=1}^{n}\left[\frac{M_i}{\bar{M}}\right]\frac{(1-f_2)s_{2i}^2}{n\tilde{m}}$$

$$(11)\quad v(\bar{\bar{y}}_3) = \frac{(1-f_1)}{n}\sum_{i=1}^{n}\left[\frac{M_i}{\bar{M}}\right]^2\frac{(\bar{y}_i - \bar{\bar{y}}_3)^2}{n-1} + \frac{f_1}{n}\sum_{i=1}^{n}\left[\frac{M_i}{\bar{M}}\right]\frac{(1-f_2)s_{2i}^2}{n\tilde{m}}$$

where

$$s_{2i}^2 = \sum_{i=1}^{m_i}\frac{(y_{ij}-\bar{y}_i)^2}{m_i - 1} = \text{within-transect variance.}$$

Cost is the other major consideration in determining an optimal sampling design. Multistage designs are nested, permitting one to estimate the optimal sampling fraction for each stage of the survey to most efficiently estimate parameters. Based on previous experience, we assumed that it was possible to collect 10 samples in the time necessary to establish a single transect (1 h) in which case for the simplest linear cost function:

$$(12)\quad C_T = nc_t + n\tilde{m}c_s$$

where C_T = total time available to conduct the survey, c_t = time to establish a transect, and c_s = time to collect each sample.

The optimal number of samples to collect per transect is then given approximately by

$$(13)\quad \tilde{m}_{\text{opt}} = \sqrt{(s_1^2 - s_2^2/\bar{M})}\cdot\sqrt{c_t/c_s}$$

where s_1^2 = between-transect variance component and s_2^2 = within-transect variance component. To achieve proportional allocation in the sampling design, we now obtain

$$(14)\quad m_i = \frac{\tilde{m}_{\text{opt}}}{\bar{M}}M_i.$$

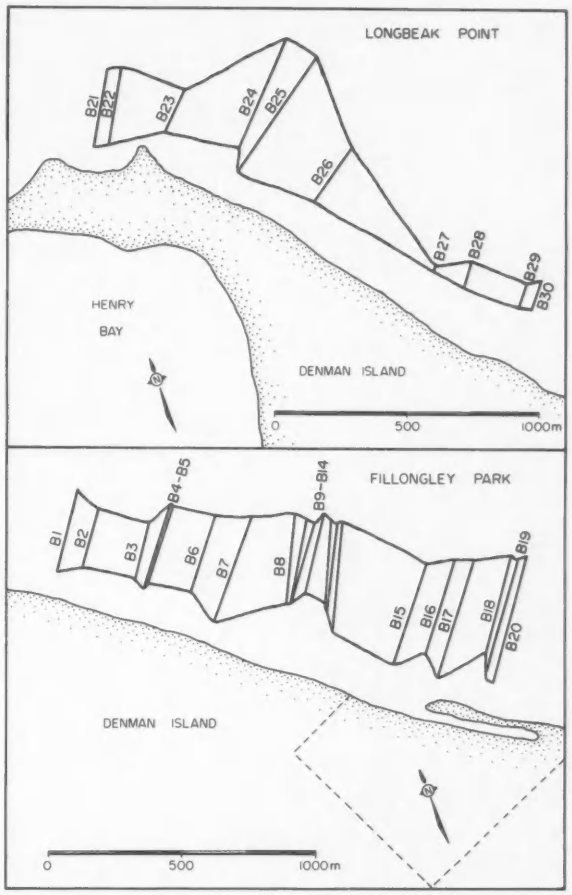

FIG. 2. Transect locations for the Fillongley Park and Longbeak Point surveys of 1981.

The optimal number of transects to sample depends on whether the survey objective is to minimize cost or variance. If, as is frequently the case, a predefined precision is desired at minimum cost, one can rearrange the estimator for the mean square error for equation 11 as found in Cochran (1977, section 11.15):

$$(15) \quad n = \left[S_1^2 - \frac{S_2^2}{\bar{M}} + \frac{S_2^2}{\bar{m}} \right] \cdot \left[V + \frac{S_1^2}{N} \right]^{-1}$$

where $V = (\text{mean} \cdot 0.25)^2$ for the standard error level. If, instead, the variance is to be minimized for a given total expenditure, one can use equation 12 to calculate the optimal number of transects to sample.

We also examined the effects on the precision of the estimates resulting from deviations from optimal values. Estimates of the relative precision of various suboptimal designs were determined as in section 10.6 of Cochran (1977). Similarly, we used equation 15 to calculate the effects on the relative standard error ($\text{SE}/\bar{\bar{Y}}$) of different sampling intensities per kilometre.

Any of equations 3, 8, or 9 may be used to estimate the average egg numbers in the spawn. When combined with confidence intervals and an estimate of the area of deposition, it becomes possible to provide managers with an estimate of the total egg deposition in that spawn with a certain level of precision. The area of deposition is delimited from vegetation maps of the area onto which transect locations are transcribed, and spot dives to define the outer edges of spawn. The total area is then integrated using a microcomputer and a large-scale map of the survey area.

The estimate of the total egg deposition may then be converted to an estimate of the number of tonnes of spawning fish required to produce the observed egg deposition. Ideally, one would like data on the age-specific fecundity and age composition of the spawning population.

In the absence of very precise estimates of the fecundity and age composition, analysis of available data indicates that there are about 200 eggs·g female weight^{-1} (Hay 1985). Hence, assuming a 1:1 sex ratio, the approximate biomass (tonnes) of spawning fish can be calculated as the product of the area covered by spawn (hectares), the density of spawn (eggs per square metre), and 10^{-4}. This is because the egg production per unit weight of fish is relatively constant, and so can be used to translate egg abundances directly into tonnes of fish.

Results

The objectives of this study were to determine the most efficient two-stage design for routine use in estimating the mean and variance for herring spawns and to provide guidelines on the requisite sampling intensity to consistently obtain estimates of egg density with acceptable precision. Table 1 indicates that there are no substantial differences between the three two-stage estimates of egg density and that from SRS. The estimator $\bar{\bar{y}}_3$ is closest to $\bar{y}_{\text{SRS}}$ but it also tends to have a larger RSE than $\bar{\bar{y}}_1$. The estimator $\bar{\bar{y}}_2$ has the highest RSE and also higher estimates of mean egg density which may reflect the difference between the average value of the m_i's and the universe estimates of average transect width ($\bar{M}$). Results obtained by combining all transects in the Strait of Georgia for each year are similar for all estimators as are the estimates of tonnes of spawning fish (Table 1). Again the results for $\bar{\bar{y}}_2$ are more different than those from the other estimators. The relative standard errors for the combined data also tend to be lower than for most of the individual areas, which suggests that between-transect variance is probably reduced by the increased sample size achieved by pooling data from adjacent areas. The relative standard error (RSE) for most areas where there is reasonable sampling effort (Tables 1, 3; 10 samples·km^{-1}) is near 0.25. By combining all data for each year this value was reduced to less than 0.20. Sampling intensity was lowest during 1978, which displays a wider range of RSE's than for the other years. The RSE's also tend to be higher for 1978. Sampling effort during 1976 is comparable with the Longbeak Point sampling of 1981 but the latter displays a much higher RSE. The 1981 and 1983 Fillongley Park surveys have the highest sampling intensities and also the lowest RSE's. For the 1981 data the marked difference in RSE for the two areas may be partially due to sampling intensity differences, since mean egg densities were similar in both areas. However, the Longbeak Point RSE is much higher than for other areas with a lower sampling intensity, suggesting some other effect.

The estimates of the within- and between-transect variance components are presented in Table 2. They indicate that the variation within a transect is universally greater than that between transects. Variance components for $\bar{\bar{y}}_2$ are always highest, while those for $\bar{\bar{y}}_1$ and $\bar{\bar{y}}_3$ are similar. For the combined data the

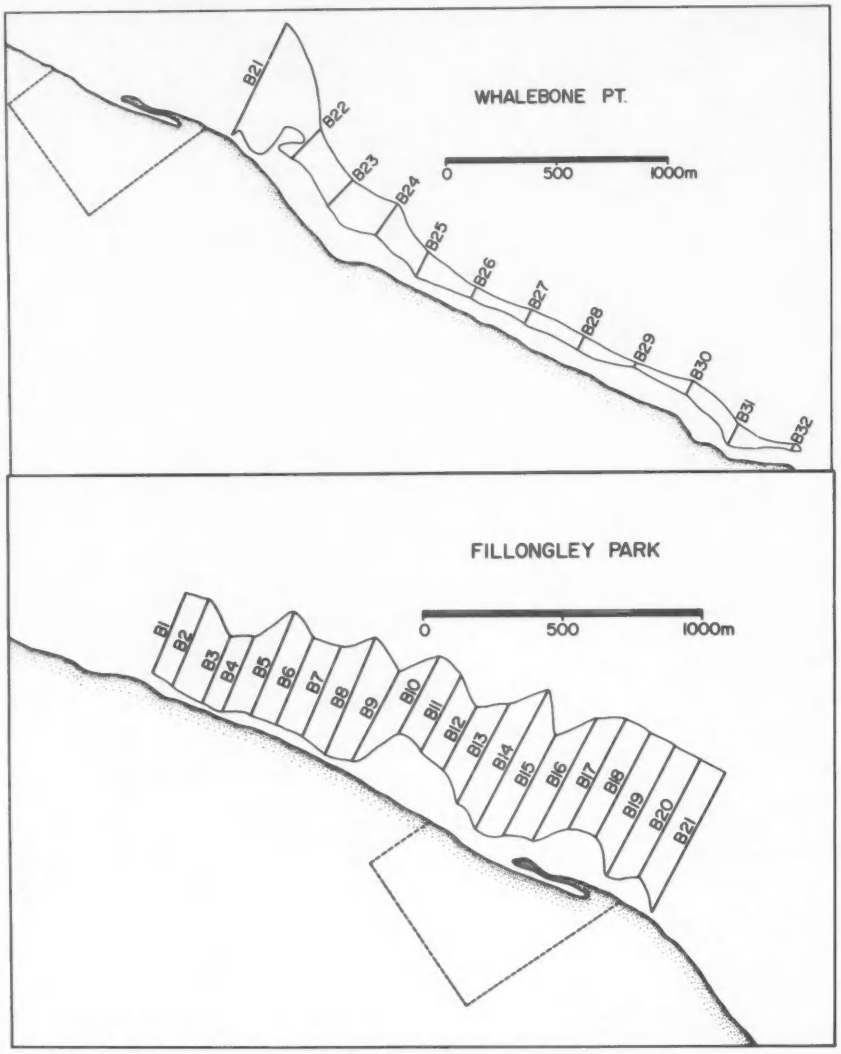

FIG. 3. Transect locations for the Fillongley Park and Whalebone Point surveys of 1983.

variance components are about equal to the average of those for the individual areas.

The relative efficiencies (RE) generally reflect the results for the variance components. The estimator for $\bar{\bar{y}}_2$ gives the greatest variance and hence the lowest RE of the three estimators. The other two are similar, with $\bar{\bar{y}}_1$ being slightly more efficient overall. In general, the two-stage designs are less efficient than SRS, usually 50–75% of SRS for $\bar{\bar{y}}_1$ and $\bar{\bar{y}}_3$, although in isolated instances they are more efficient. For the combined data, however, they are all less than 50% as efficient as SRS.

The sampling rates and optimal sampling fractions are presented in Table 3. The estimated optimal sampling fractions ($\tilde{m}/\tilde{M}$) are quite consistent between areas and years at 2–6%. The results for the combined data indicate that sampling either 3 or 4 stations per 100 m of transect is optimal. Examining the relative variance for the combined data (Fig. 4) clearly indicates that sampling below 2% results in rapid increases in variance, while the curve for the optimum is quite flat at higher sampling rates. Therefore, sampling rates of 0.02–0.05 should result in variance estimates no more than 10% greater than would be obtained with the optimal sampling rate.

The estimates of the optimal number of transects to sample per kilometre of spawn are more variable. They range from 2 to 30 but are usually fewer than 6. For the combined data they range from 2 to 6 but probably should be near the lower end. Figure 5 displays the RSE (SE/$\bar{\bar{Y}}$) for the $\bar{\bar{y}}_3$ estimator and various numbers of transects per kilometre. To achieve the desired RSE for 1976 and 1978 data clearly requires no more than 2 transects·km^{-1}. These results are probably too low, while those for 1981 appear to be too high, as will be discussed below. The 1983 data indicates that 3 transects·km^{-1} are optimal, while 4 should bring the RSE down to 0.20. Thus, transects spaced every 250–400 m along a spawn should result in estimates of the mean egg density with the desired precision.

TABLE 1. Estimated mean egg number·m^{-2} ($\times 10^{-3}$) and its relative standard error (RSE) for simple random sampling (SRS) and three two-stage designs. An estimate of tonnes of fish from the egg deposition is also presented.

									Approximate tonnes of fish			
	$\hat{y}_{SRS}$	RSE	$\bar{\bar{y}}_1$	RSE	$\bar{\bar{y}}_2$	RSE	$\bar{\bar{y}}_3$	RSE	$\hat{y}_{SRS}$	$\bar{\bar{y}}_1$	$\bar{\bar{y}}_2$	$\bar{\bar{y}}_3$
1976												
Icarus Pt.	1105.0	0.11	1209.8	0.12	982.2	0.22	1099.7	0.11	7 901	8 651	7 023	7 864
Nanoose Bay	942.2	0.10	868.4	0.21	1135.9	0.37	992.9	0.24	2 950	2 719	3 557	3 109
1978												
Qualicum R.	436.6	0.22	357.7	0.39	496.2	0.64	521.9	0.24	1 292	1 059	1 469	1 545
L. Qualicum W.	358.9	0.19	297.0	0.26	488.5	0.43	364.6	0.26	1 816	1 503	2 472	1 845
L. Qualicum R.	98.2	0.48	96.9	0.03	204.8	0.46	98.0	0.05	198	196	414	198
Qualicum Beach	795.7	0.35	730.6	0.36	820.8	0.15	672.3	0.45	1 790	1 644	1 847	1 513
French Creek	548.2	0.13	584.3	0.08	855.1	0.35	568.1	0.15	1 349	1 437	2 104	1 398
Parksville Bay	382.9	0.26	413.8	0.25	645.8	0.41	421.5	0.53	1 218	1 316	2 054	1 340
Northwest Bay	928.0	0.18	1012.7	0.22	1039.9	0.19	932.7	0.25	3 461	3 777	3 879	3 479
1981												
Fillongley Pk.	246.4	0.09	266.8	0.15	238.0	0.12	248.0	0.13	1 770	1 917	1 710	1 782
Longbeak Pt.	279.1	0.31	186.2	0.41	360.2	0.57	289.6	0.51	1 139	968	1 488	1 197
1983												
Fillongley Pk.	181.0	0.07	189.0	0.12	184.8	0.10	182.5	0.11	1 390	1 452	1 420	1 402
Whalebone Pt.	119.3	0.23	127.0	0.23	78.3	0.38	113.8	0.24	435	463	285	415
Combined												
Gulf 1976	1023.6	0.07	996.4	0.13	957.5	0.22	1052.4	0.10	10 524	10 245	9 845	10 821
Gulf 1978	563.7	0.10	569.3	0.15	762.3	0.14	550.6	0.18	12 210	12 331	16 512	11 926
Gulf 1981	252.9	0.10	241.8	0.15	290.0	0.18	258.4	0.17	2 862	2 737	3 282	2 925
Gulf 1983	173.7	0.07	167.7	0.11	217.0	0.14	175.4	0.13	1 967	1 899	2 457	1 987

TABLE 2. Estimates of the between (SSB) and within (SSW) transect variance components and the relative efficiencies (RE) of the three two-stage designs.

	$\bar{\bar{y}}_1$			$\bar{\bar{y}}_2$			$\bar{\bar{y}}_3$		
	SSB	SSW	RE[a]	SSB	SSW	RE[a]	SSB	SSW	RE[a]
1976									
Icarus Pt.	133 162	1 212 653	0.63	286 897	1 052 002	0.29	85 573	1 052 002	0.98
Nanoose Bay	321 295	591 242	0.28	1 728 050	649 357	0.05	581 300	649 351	0.15
1978									
Qualicum R.	76 835	47 144	0.47	409 891	71 564	0.09	64 508	71 564	0.56
L. Qualicum W.	30 834	71 116	0.75	222 482	140 453	0.10	46 012	140 453	0.50
L. Qualicum R.	12	18 587	247.89	17 423	41 893	0.26	34	41 893	93.73
Qualicum Beach	135 956	503 396	1.15	28 414	510 628	5.46	182 929	501 628	0.85
French Creek	9 649	92 919	2.24	367 448	155 780	0.06	29 305	155 780	0.74
Parksville Bay	54 523	267 340	0.89	343 305	428 320	0.14	254 136	428 320	0.19
Northwest Bay	382 023	1 119 549	0.56	313 421	1 071 660	0.68	452 394	1 071 660	0.47
1981									
Fillongley Pk.	30 468	144 696	0.34	16 940	124 761	0.60	22 054	124 761	0.47
Longbeak Pt.	53 137	260 130	1.26	374 852	616 768	0.18	197 438	616 768	0.34
1983									
Fillongley Pk.	10 144	59 810	0.33	7 907	56 450	0.42	8 126	56 450	0.41
Whalebone Pt.	9 296	34 996	0.86	9 948	22 678	0.81	7 984	22 678	1.01
Combined									
Gulf 1976	266 313	824 271	0.35	727 436	825 791	0.13	169 817	825 791	0.54
Gulf 1978	212 204	408 430	0.48	364 038	490 443	0.28	295 585	490 443	0.35
Gulf 1981	37 298	181 900	0.48	75 974	249 019	0.24	57 182	249 019	0.32
Gulf 1983	10 440	51 280	0.41	31 612	66 034	0.14	15 516	66 034	0.28

[a]Variance for SRS/variance for two-stage sampling.

TABLE 3. Estimated optimal sampling designs required to attain a standard error of 25% of the mean assuming a two-stage design with unequal primaries (ratio estimator).

	Spawn length (km)	Average spawn width (m)	Universe average width $\bar{M}$[a]	Total area (ha)	Transects sampled	Transects·km^{-1}	Average samples·transect^{-1}	Average samples·km^{-1}	Total samples	Optimal sampling fraction [$\bar{m}/\bar{M}$]	Optimal transects·km^{-1}
1976											
Icarus Pt.	1.70	376	420.6	71.51	6	3.5	14	50	85	0.03	2
Nanoose Bay	2.97	179	156.6	31.31	10	3.4	8	29	85	0.02	7
1978											
Qualicum R.	2.97	95	99.7	29.60	4	1.3	3	4	13	0.03	2
L. Qualicum W.	4.46	152	113.5	50.60	5	1.1	4	4	19	0.05	2
L. Qualicum R.	2.27	186	89.0	20.20	2	0.9	4	4	8	—	—
Qualicum Beach	1.68	115	133.9	22.50	2	1.2	4	5	8	0.04	6
French Creek	2.04	182	120.6	24.60	4	2.0	4	8	16	0.06	2
Parksville Bay	2.02	241	151.4	31.80	5	2.5	4	11	22	0.03	16
Northwest Bay	3.00	139	124.3	37.30	8	2.7	4	11	32	0.04	5
1981											
Fillongley Pk.	2.00	345	359.3	71.85	20	10.0	14	140	279	0.02	6
Longbeak Pt.	2.00	257	206.6	41.31	9	4.5	8	35	70	0.03	30
1983											
Fillongley Pk.	2.00	389	384.1	76.81	21	10.5	18	189	378	0.03	4
Whalebone Pt.	2.92	86	124.8	36.44	11	3.8	5	17	51	0.04	6
Combined											
Gulf 1976	4.67	253	277.9	102.82	16	3.4	11	36	170	0.03	2
Gulf 1978	18.44	163	117.5	216.60	30	1.6	4	6	118	0.04	2
Gulf 1981	4.00	318	282.9	113.17	29	7.3	12	87	349	0.03	6
Gulf 1983	4.92	285	230.2	113.25	32	6.5	13	87	429	0.03	3

[a]Total area/spawn length.

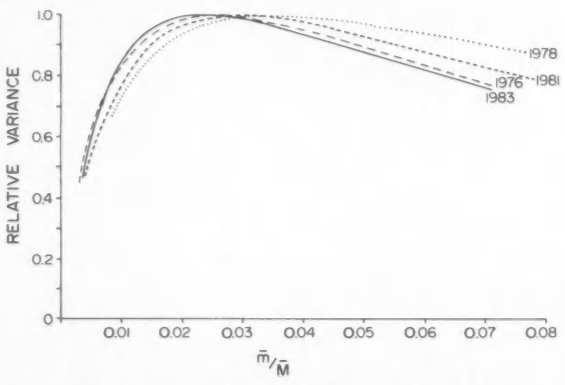

FIG. 4. Relationship between the sampling intensity ($\bar{m}/\bar{M}$) and the resulting variance relative to that at the optimal sampling intensity for the combined data in all years using the ratio estimator.

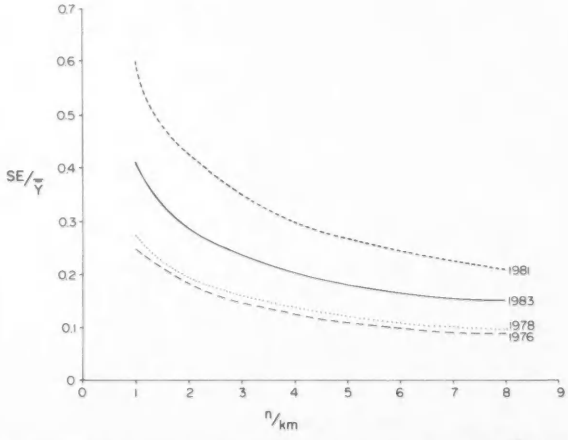

FIG. 5. Relationship between the relative standard error (SE/$\bar{Y}$) and the number of transects·km^{-1} for the combined data in all years using the ratio estimator.

Discussion

The design of any sampling scheme rests on two basic considerations: the cost of sampling and the precision of the estimates required. Depending on which element is more important to the researcher, the best survey design may be quite different. In most instances, the researcher will opt for the best estimates of precision possible with but vague consideration of the costs involved. In the final analysis it will be the total cost either implicit or explicit that determines the extent and intensity of the sampling survey. In many cases practicality of sampling has an important impact on the type of sampling undertaken. In this survey it is much simpler to lay down transect lines and sample along them than to take an equivalent number of samples at random. Divers would be going up and down continuously and there would be difficulty in defining the boundaries of spawn as well as in ensuring that sampling was confined to the area of spawn deposition. These considerations should, of course, enter into the decision of the sampling scheme through

their effects on survey costs, but it is exceedingly difficult to make an accurate assessment of such factors.

Instead, we have accepted the fact that our sampling scheme falls within the realm of the two-stage design and treated the data accordingly. We have, however, tried to optimize our sampling within the framework of the two-stage design to ensure that the sampling is done as efficiently as possible given the logistic constraints of the survey.

The main questions that remain are which of the two-stage designs provides the most efficient estimate of precision and how much sampling is required to achieve the desired precision in the estimate of mean egg density.

The three estimators for the mean differ only in how they weight the average egg density obtained from each transect. As such they should agree quite closely, and only $\bar{\bar{y}}_2$ yields consistently aberrant results. This occurs most markedly where the individual transect widths do not seem to be good estimators of the universe average spawn width ($\bar{M}$, Table 3). This is particularly true for large areas of spawn with relatively few transects in them. The estimator $\bar{\bar{y}}_1$ is questionable because it weights all transects equally although there may be widely different sample numbers for different transects. The preferred estimator for the mean appears to be $\bar{\bar{y}}_3$, since it weights the mean by the transect size and, assuming there is reasonable sampling intensity, should provide accurate estimates of $\bar{M}$ and so have minimal bias.

Similarly, the estimates of the variance (i.e. the RSE and RE) are comparable for the three estimators although that for $\bar{\bar{y}}_2$ is again aberrant and higher than for the other two (Tables 1, 2). This again appears to result from the discrepancy between the average of the M_i's and the universe estimate of mean transect width, $\bar{M}$ (Table 3). In areas where the two agree quite closely the estimate of mean egg density and its variance is comparable with that from $\bar{\bar{y}}_1$ and $\bar{\bar{y}}_3$. However, this estimator ($\bar{\bar{y}}_2$) seems to be quite sensitive to even small differences between the average transect width and the universe estimate, $\bar{M}$. For the combined data with the increased sample size the mean M_i should be a better estimate of $\bar{M}$, which appears to be the case. However, the estimates of $\bar{\bar{y}}_2$ and its variance are markedly different from those for the other two estimators for all four years of combined data. Thus, the estimator $\bar{\bar{y}}_2$ appears to be too unstable to recommend it for routine use in this two-stage design.

Surprisingly, there were no clear effects detectable as the result of varying sampling intensities. The combined data had a lower RSE than most of the individual areas but it was not markedly lower. Some areas such as Longbeak Point in 1981 were anomalously high. In this instance the vegetation appears to be very patchy and the data for this area contained a large number of empty quadrats. Curiously, the 1976 and 1978 data do not have anomalously low RSE values, since in both these years the sampling was done systematically but did not include any zero quadrats, i.e. either the sampling concentrated on obtaining "representative" samples purposely avoiding bare areas (or zero quadrats) or the information for the empty quadrats was not recorded. One would expect then that this data should be less variable than the later information which also sampled the bare or patchy zones in the spawn. In part this is masked by density differences because the variance appears to vary with the mean. Also it is masked by the nature of the estimator, since because we are sampling so little of the spawn (i.e. n/N negligible) the within-transect variance component has only a small effect on the total variance. However, ignoring zero quadrats in the 1976 and 1978 data should also lower the between-transect variance and consequently the RSE. For the $\bar{\bar{y}}_3$ estimator this could be the case, since the RSE is comparable with that in the 1983 data where the sampling was almost twice as intense (transects per kilometre, Table 3). Examining only the 1981 and 1983 data shows a clear effect of sampling intensity. The intense sampling at Fillongley Park in both years yields a lower RSE than the lighter sampling at Longbeak Point and Whalebone Point.

Relative efficiencies do not differ much between estimators but they are usually less than for SRS. The reasons for this are not particularly clear. Either the within- or between-transect variances are much too large. The advantage of complex sampling designs is that it permits one to reduce variation due to subsets of data that differ markedly in magnitude from one another by combining them and assessing their variation separately. In this case, samples along a transect are treated as a unit, i.e. similar in magnitude. However, herring spawn may not really be deposited in this fashion. In fact, the available evidence indicates that most of the eggs are deposited near the spring low tide level (Haegele et al. 1981). A transect across the spawn then often samples markedly lower egg densities at the inner and outer edges of the spawn. This would tend to inflate the within-transect variance. However, it should be noted that in many instances spawn density is very uniform along an entire transect. Heterogeneity in deposition can also occur between transects, as fish appear to move parallel to shore when they spawn and will stop and start in response to unknown environmental factors. Also there are physical discontinuities and bare areas in the vegetation substrate which can all result in marked differences in mean egg density between adjacent transects. In essence, sampling is concentrated perpendicular to shore, while spawn deposition is largely dependent on the plant community which is structured parallel to shore and largely affected by the water depth and available physical substrate.

As indicated earlier, if sampling intensity is fairly low it is the between-transect variance that determines the magnitude of total variance. Therefore, as is evident in Table 1, combining data within years should increase sample size and reduce between-transect variance in most instances. However, it also means that unless the individual transect means are at least as homogeneous as the total group of samples from a spawn, the two-stage design will be less efficient than SRS. Because of the heterogeneity of most shorelines, plant community composition and density change and thereby the available spawn substrate. Consequently, the spawn density will also be affected making it exceeding unlikely, except in unusual circumstances, that the spawn will be so homogeneous that two-stage sampling will be as efficient as SRS.

The estimates of optimal sampling design using $\bar{\bar{y}}_3$ indicate uniformly for all spawns, both individually and combined, that about 2–5% of the transect should be sampled. Unfortunately there were no very narrow transects in this survey, since this scheme implies no sampling at the lower level for transects under 50 m and only 1 sample for transects less than 100 m wide, making it impossible to estimate the within-transect variance for these spawnings.

One would expect the narrower spawns to also be more heterogeneous, since they tend to occur in areas of steeper slope where the vegetation community changes rapidly over a shorter distance. This could have a major impact on the variance component estimates and thereby on the estimate of $\bar{m}_{opt}$. This effect is evident in the data for the relatively more narrow spawn at Whalebone Point in 1983. The higher $\bar{m}_{opt}$ for this area

suggests a larger s_1^2/s_2^2 ratio than for some of the wider areas. There it is constant at 3–4 and the spawn appears to be more homogeneous. Nevertheless, the results are quite robust, as is evident from Fig. 1 and equation 13 where it may be seen that the cost ratio would have to change drastically to introduce a significant change in the optimal subsampling fractions. Because of the robustness of the results and the apparent lability of the variance ratio with respect to spawn width, we recommend that sampling be done at the higher rate for any spawns narrower than 100 m and at the same or lower rate for wider spawns.

The estimates for the optimal number of transects to sample per kilometre are less clearly defined. Based on individual areas in 1976 and 1978 the estimates range from 2 to 16 but generally less than 6 and 2 for the combined data. Because of the problems with the sampling not being representative here, i.e. no bare areas being sampled, this must be considered as a lower limit to sampling frequency. The 1981 data suggest 6 and 30 transects·km^{-1} individually and 6 for the combined data, which again is influenced by the apparently aberrant Longbeak Point sampling. Looking only at the Fillongley Park data from 1981 and 1983 we get a range of 4–6 which is consistent given the same level of sampling in the two years leading to virtually identical variance estimates. The combined 1983 data leads to a comparable estimate of 3 transects·km^{-1}. The sampling envelope in Fig. 5 leads to the general conclusion that no fewer than 2 and no more than 4 transects·km^{-1} based on the 1983 data be sampled to obtain the desired precision in egg density estimates. In other words, transects every 250 to 400 or 500 m apart should adequately sample all herring spawns in the Strait of Georgia. There are of course some other considerations such as weather and spawn timing that may influence the sampling rate. For example, there are about 2 wk between spawn deposition and hatching in which to complete the spawn survey. In the Strait of Georgia, spawning is slightly staggered in time so that spawnings on the mainland and the Vancouver Island shoreline may occur almost simultaneously or 1 or 2 wk apart. Depending on the synchronicity of the spawning there may be more or less time available in which to survey the first spawn to enable the completion of the survey of the second prior to its hatching. Consequently, the optimal sampling rate would be altered by time constraints.

A consideration not dealt with in detail here that we attempted experimentally in the 1981 study is random versus systematic subsampling. In effect, it was "pseudo" random sampling where we randomly selected 3 or 4 quadrat locations per 100 m of transect length because there was no a priori way to determine total transect length, since it is impossible to know the exact limits of the spawn distribution prior to sampling it. Nevertheless it is interesting that there were no detectable differences in either the variance estimates or optimal design estimates from the 1981 and 1983 surveys, particularly the repeat work at Fillongley Park.

It was our experience that the random sampling even along transects was exceedingly difficult to implement, only partly because it was done underwater. In general, there seem to be sufficient logistical exigencies in most field sampling situations to warrant some form of systematic sampling. In this case, transects and samples are actually located randomly with respect to the underlying spawn distribution so the estimators described herein are still appropriate. The same approach has been adopted in the sampling of various commercial fisheries where vessels arrive in port at random but are sampled in a systematic fashion to obtain a random sample (Quinn et al. 1983; Sen 1984).

In summary, we have been able to demonstrate that a two-stage sampling design can be used to provide estimates of the mean egg density with the desired precision. Data obtained by an apparently biased procedure in 1976 and 1978 nevertheless provided a useful estimate of the optimal sampling fractions for designing the 1981 survey. Although aberrant results in the Longbeak Point area in 1981 prevented the demonstration of a generally applicable sampling design, the 1983 data corroborated most of the 1981 results. The hypothesis that an optimal sampling scheme of 2–5% of each transect depending on width and 3 or 4 transects·km^{-1} is universally adequate to meet our sampling needs should be tested with future surveys in other areas.

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Suitability of Vertical Slot and Denil Fishways for Passing North-Temperate, Nonsalmonid Fish

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A vertical slot fishway and two Denil fishways (of 10 and 20% slope) built into a weir on the Lesser Slave River (55°18'N, 115°45'W) were studied from May 12 to June 25, 1984, to determine how effectively these designs pass north-temperate, nonsalmonid fishes. Thousands of spottail shiner (*Notropis hudsonius*), substantial numbers (>100) of northern pike (*Esox lucius*), longnose sucker (*Catostomus catostomus*), white sucker (*Catostomus commersoni*), immature yellow perch (*Perca flavescens*), and lesser numbers of burbot (*Lota lota*), adult yellow perch, lake whitefish (*Coregonus clupeaformis*), and trout-perch (*Percopsis omiscomaycus*) ascended the fishways. Walleye (*Stizostedion vitreum*) and goldeye (*Hiodon alosoides*), although probably moving extensively through the river, did not use the fishways. Although high water levels allowed most fish to surmount the weir, of those that chose the fishway, pike strongly preferred to ascend the Denil fishways and the two sucker species preferred to ascend the vertical slot. Therefore, a combination of several different fishways may be required for the most efficient passage of a wide variety of species. Plasma glucose and lactate measurements on pike revealed that ascending the Denil fishways was only moderately stressful for these fish.

Du 12 mai au 25 juin 1984, on a étudié une passe verticale à chicanes et deux passes de type Denil (ayant une pente de 10 et 20 %) construites dans un barrage situé sur la petite rivière des Esclaves (55° 18' N, 115° 45' O) pour déterminer avec quelle efficacité ces dispositifs permettent le passage d'espèces de poissons de la zone tempérée nord qui ne font pas partie des salmonidés. Des milliers de queues à tache noire (*Notropis hudsonius*), un nombre important (>100) de grands brochets (*Esox lucius*), de meuniers rouges (*Catostomus catostomus*), de meuniers noirs (*Catostomus commersoni*), de jeunes perchaudes (*Perca flavescens*) et un moins grand nombre de lottes (*Lota lota*), de perchaudes adultes, de grands corégones (*Coregonus clupeaformis*) et d'omiseos (*Percopsis omiscomaycus*) ont gravi les échelles à poissons. Le doré jaune (*Stizostedion vitreum*) et la laquaiche aux yeux d'or (*Hiodon alosoides*), bien qu'ils se déplacent probablement beaucoup dans la rivière, n'ont pas utilisé ces passes. Bien que le niveau élevé de l'eau ait permis à la plupart des poissons de franchir le barrage, parmi ceux qui ont choisi de gravir l'échelle à poissons, le brochet a préféré de beaucoup utiliser les passes « Denil », tandis que les deux espèces de meunier ont choisi plutôt la passe verticale. Par conséquent, il se peut qu'on doive utiliser plusieurs types de passes pour que le passage d'une grande variété d'espèces se fasse le plus efficacement possible. Des mesures de la teneur en glucose et en lactate du plasma prises chez les brochets ont révélé que la montée des passes « Denil » n'a provoqué qu'un stress modéré chez ces poissons.

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In North America, facilities allowing fish to pass barriers in waterways have traditionally been designed for various species of salmonids (Collins et al. 1962, 1963; Weaver 1963). However, the rivers of Canada's northern plains and boreal regions contain many other species of migratory fish. These rivers have seen increased construction of dams and weirs for water flow regulation, and environmental legislation requires that passage around obstructions be provided for all migratory species. Seasonal or spawning related movements have been documented for northern pike (*Esox lucius*) (Carbine 1941; Schultz 1955), several catostomid species (Harris 1962; Geen et al. 1966), walleye (*Stizostedion vitreum*) (Crowe 1954; Rawson 1956), goldeye (*Hiodon alosoides*), and mooneye (*Hiodon tergisus*) (Paterson 1966; Munson 1977; Trautman 1981). Unfortunately, the scientific literature contains little information on the swimming ability of these species (Beamish 1978) and no information on their use of fishways.

Fishway designs are of three main types: the step and pool, the vertical slot, and the Denil (Clay 1961; Bell 1973; Jens 1973; Rajaratnam et al. 1984). Water level greatly influences the operation of the step and pool fishway which therefore requires frequent adjustment in rivers with widely fluctuating water flows. The vertical slot and Denil designs require less maintenance because they operate effectively over a wide range of water levels. Substantial savings in construction costs can be achieved by decreasing the length of the fishway (thus increasing the fishway's slope and water velocity) to the minimum that still allows efficient fish passage. However, extensive study of

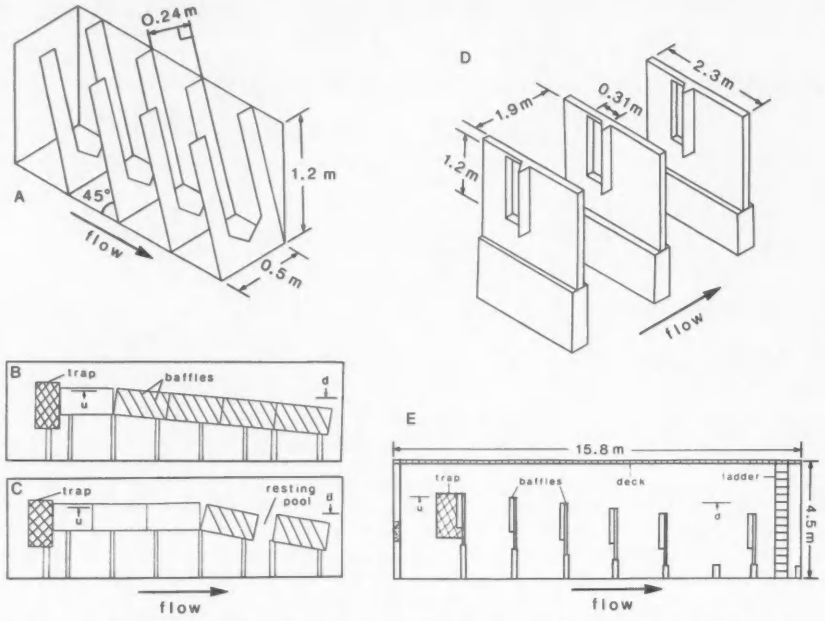

FIG. 1. Scale drawings and dimensions of the Denil (A) and vertical slot (D) fishways. Also shown are side-views of the 10% slope Denil (B), 20% slope Denil (C), and vertical slot (E) fishways as they appear inside the enclosure. The positions of the fishway traps are shown, as are the median upstream and downstream water levels (denoted by u and d, respectively).

how hydraulic characteristics affect fish passage have been performed only for the step and pool design and then only for salmonids (Collins et al. 1962, 1963; Weaver 1963).

The purpose of the present study was to evaluate the effectiveness of different fishway designs in passing various north-temperate, nonsalmonid fish during their seasonal movements. The study was performed at a new fish passage facility built by the Alberta government in a weir on the Lesser Slave River (55°18′N, 115°45′W). This facility consists of a combination of three different fishways (one vertical slot, a 10% slope Denil, and a 20% slope Denil) located side by side inside a single enclosure. Thus, the fishways were studied under natural operating conditions and the three designs could be validly compared because they were subjected to an identical hydraulic head.

We also measured the plasma glucose and lactate levels of northern pike to determine how stressed they were by exercise performed while ascending the Denil fishways. Previous studies showed that severe exercise can stress fish to the point of mortality (Black 1958) and cause great elevations in the plasma glucose and lactate levels of pike (Schwalme and Mackay 1985a).

Materials and Methods

The fishways studied were located on the Lesser Slave River about 1 km below its exit from Lesser Slave Lake from whence the river flows southeastward about 60 km to join with the Athabasca River. The fishways were intended to let migrating fish bypass a concrete weir that is part of a lake water level control structure. All three fishways (designs in Fig. 1) were inside a corrugated steel enclosure that had overhead decking made of steel grates (Fig. 2).

The two Denils were situated side by side and together occupied one half of the enclosure. Both Denils consisted of a rectangular sloping trough into which were slotted baffles that had V-shaped bottoms (Fig. 1A). While allowing water to flow at high velocity near the surface, the baffles create turbulence at the bottom which slows water velocity sufficiently for fish to ascend. At their upstream end both Denil fishways contained a horizontal flume which was without baffles. The sloping, baffled portion of the 20% Denil contained a resting pool and was much shorter than that of the 10% Denil (Fig. 1B, 1C).

The vertical slot fishway consisted of a series of six baffles each containing a tall narrow slot through which water flowed (Fig. 1D). Much of the water's energy is dissipated as it flows in a circular pattern through the bays between the baffles. Triangular-shaped deflectors located next to the vertical slots aid in maintaining circular water movement.

Accurate rating curves of water elevation versus weir and fishway water velocities were developed from extensive study at the University of Alberta Hydraulics Laboratory (see Rajaratnam et al. 1984 for Denil fishways). During the study period, daily water levels at the upstream and downstream ends of the fishways were recorded as the average of at least two readings per day. The median of the daily water levels and upper and lower limits which contain 75% of the daily water levels between them (37.5% above and below the median) were calculated. These water levels were used to determine weir and fishway water velocities (Table 1) from the rating curves.

In traversing the weir, fish had to swim a distance of about 3.0 m against the velocities in Table 1. The total distance of high

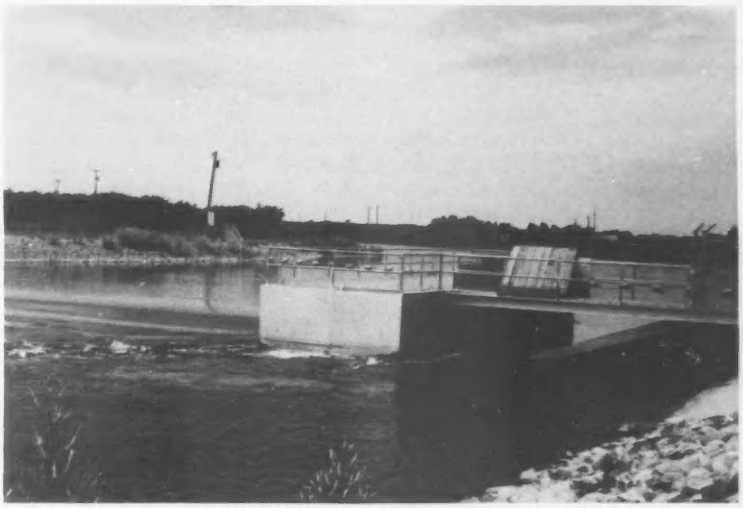

FIG. 2. Enclosure housing the three fishways. The crest of water to the left of the fishway enclosure shows the location of the concrete weir. Note the spillway between the right (east) wall of the fishway enclosure and the east bank of the weir.

velocity flow through each of the vertical slots was about 50 cm. During the study period the lower third of all fishways (including the lower four baffles of the vertical slot fishway) were submerged because water levels downstream of the weir were higher than normal (Fig. 1). This meant that the total distance of high velocity flow was only about 100 cm (i.e. two slots) in the vertical slot fishway, 8.3 m in the 10% Denil, and 9.3 m in the 20% Denil. Under nonflooded conditions fish have about 3.0 m (i.e. six slots × 0.5 m) of high velocity flow to swim through in the vertical slot fishway and 12.3 m in the Denils (this includes the flume and baffled portions).

Movement of fish through the fishways was monitored with traps placed flush against the fishway exits as shown in Fig. 1. These traps consisted of a rectangular frame made of 1.3-cm steel bars and covered (on all sides except that facing the fishway) with screening. The trap in the vertical slot fishway was covered with 0.6-cm wire mesh. Five-centimetre plastic mesh was used to cover the traps in the Denil fishways because the smaller mesh would decrease fishway water flow and thereby invalidate comparisons between the Denil and vertical slot fishways. Small mesh was satisfactory on the trap in the vertical slot fishway because this fishway had much less water flow than the Denils. The vertical slot trap measured 152 cm high, 102 cm long, and 102 cm wide. Both Denil traps were 203 cm high, 102 cm long, and 64 cm wide.

The vertical slot fishway began operation on May 11, 1984, and the Denil fishways on May 18. A trap was installed in the vertical slot fishway on May 12 but operated only occasionally until May 18. The Denil traps were installed on May 21. Traps were usually checked every 3 h. All three traps extended about 64 cm below the exits of the fishways and fish appeared to remain in this area of quiet water rather than swim back down the fishway. However, on June 6 we installed funnels on all traps to ensure that fish could not escape. Dacron netting of 3.8-cm mesh was used to make these funnels which consisted of two rectangular panels that came together in a V as they extended into the trap from the exit of the fishway. At the tip of the V the two panels were loosely tied together at intervals of about 20 cm. Fish could enter the traps by pushing apart the panels at the tip of the V, but once inside would have greater difficulty in finding and escaping through the narrow opening. The ability of fish to escape our traps was tested on three occasions by placing three to five pike or suckers (*Catostomus* spp.) in each trap overnight (12 h). No fish ever escaped during this interval which was the longest interval between trap checks in our study.

TABLE 1. Fishway and weir water velocities ($m \cdot s^{-1}$) during the study. Velocities are shown for the median daily water levels and the upper and lower limits which contain 75% of the daily water levels between them (i.e. 37.5% on either side of the median).

	Lower limit	Median	Upper limit
Floor of both Denil fishways	0.30	0.42	0.46
Surface velocity in 10% Denil	1.60	2.25	2.38
Surface velocity in 20% Denil	2.05	2.85	2.95
Avg. velocity in flume of 10% Denil	0.52	0.76	0.80
Avg. velocity in flume of 20% Denil	0.75	1.07	1.13
Vertical slot fishway (through slots)	0.23	0.60	0.68
Centre of weir	1.50	1.75	1.80
Side of weir	1.15	1.30	1.35

After the funnels were in place the traps were checked at 09:00 and 21:00 each day until 12:00 on June 25, 1984, when the study ended. Nighttime operation of the traps was occasionally interrupted when beavers entered them and chewed through the plastic mesh. After May 23, fish passage through the fishways virtually ceased and many fish were seen swimming through a small spillway located between the fishways and the east bank of the weir (see Fig. 2). Therefore, on May 28, fish passage through the spillway was blocked with a sheet of plywood and this was successful in getting modest numbers of fish to use the fishways again.

We also spent a total of 24 h counting fish as they swam over the weir to determine the number of fish that used this route rather than the fishways to proceed upstream. The weir was observed from either the west bank or the fishway deck (sometimes from both locations) for 1–3 h on 11 different dates between May 13 and June 23 (whenever the water was sufficiently clear). The count obtained is probably an underestimate because most of the time, viewing conditions did not allow the entire weir to be observed. A total of 6.5 h (on May 27, June 22, and June 23 when the plywood barrier was absent) was spent counting fish passage through the spillway.

From May 12 to 25, the river's temperature was fairly constant at 9°C and thereafter climbed irregularly to 17–18°C by June 25.

Beginning June 4, fish were captured from the river by seining to compare their species composition with that of the trap-caught fish. This was done to determine whether certain species of fish were ascending the fishways in numbers disproportionate to their abundance in the river. Fish were collected from immediately below the fishway's entrance and from a short (100 m long), shallow bay that joined the river about 100 m downstream of the weir. Each location was seined for several hours, usually once a day, the time (morning or afternoon) being altered from day to day.

Northern pike are known to congregate below weirs and fishways on other Alberta rivers (Nelson 1983; Fernet 1984). However, it is not known whether pike aggregate there because they have difficulty in traversing these structures or because they are attracted to the structures by an abundance of food organisms. We therefore stomach-pumped seine-caught pike (according to Crossman and Hamilton 1978) on several occasions to determine the extent of their feeding.

Because fish struggle when removed from the fishway traps for blood sampling, plasma glucose and lactate measurements obtained in this way may not accurately indicate the levels of exercise and stress associated with ascending the fishway. We attempted to overcome this problem by sampling blood from three groups of pike to quantify the effect of the sampling procedure on these biochemical parameters. Group 1 consisted of pike that had volitionally ascended one of the Denil fishways in the previous 3 h. After a 3-h set, the traps were lifted from the water, the fish quickly removed, and blood (1 mL) drawn from a branchial artery. Previous work (Schwalme and Mackay 1985a, 1985b) indicated that after exercise, blood lactate levels of pike are substantially elevated within 5 min and blood glucose within 30 min, and both levels remain elevated for at least 3 h. A second group of pike was sampled to determine how high blood lactate and glucose levels were elevated by the sampling procedure. These fish were seined from the river, allowed to recover overnight (12 h) in a trap set in one of the Denil fishways, and sampled exactly as group 1. Twelve hours is sufficient for blood lactate and glucose levels of pike to return to normal after vigorous struggling (Schwalme and Mackay 1985b). Our reasoning was that if the plasma glucose and lactate levels were higher for group 1 than for group 2, the excess should be due to exercise performed in ascending the fishway rather than to struggling during sampling. Time (mean ± SD, $n = 12$) between start of the fish's struggling and completion of blood sampling was 0.9 ± 0.2 min for group 1 and 1.3 ± 0.5 min for group 2. We also attempted to measure resting plasma lactate and glucose levels by sampling pike that were anaesthetized without struggling after having rested for 12 h in a 400-L tank. Unfortunately, these fish had abnormally high plasma lactate levels and therefore the data were omitted. However, loss of this data is not critical because satisfactory measurements of resting blood lactate and glucose levels are available in the literature (Schwalme and Mackay 1985a, 1985b).

Blood sampled from fish was immediately centrifuged and the plasma kept frozen until analysed. Glucose was measured with a commercial kit (Sigma Chemical Co., St. Louis, MO, Tech. Bull. 510) employing the glucose-oxidase, peroxidase method of Raabo and Terkildsen (1960). The method of Barker and Summerson (1941) was used to measure lactate.

Results

Under the observed flow conditions, 10 species (most represented by a large size range) were able to ascend one or more of the fishways (Table 2). Northern pike, longnose sucker (*Catostomus catostomus*), and white sucker (*Catostomus commersoni*) were predominant among the large fish, while lake whitefish (*Coregonus clupeaformis*), cisco (*Coregonus artedii*), and burbot (*Lota lota*) were caught only in small numbers. Spottail shiner (*Notropis hudsonius*) were numerically the most abundant fish. Spottail shiner, juvenile perch (*Perca flavescens*), and trout-perch (*Percopsis omiscomaycus*) were caught only in the vertical slot trap because the large mesh on the other traps allowed them to swim through. Nevertheless, spottail shiner were also observed to ascend the 10 and 20% Denil fishways in considerable numbers.

The high water levels also allowed fish to swim over the weir and through the spillway located east of the fishway enclosure. Visual observations indicated that many more pike and sucker swam over the weir than through the fishways (Table 3). Assuming that twice as many large fish swam over the weir between 09:00 and 21:00 than vice versa (as occurred for fishway ascent, Table 4), we estimate that about 3470 fish traversed the weir during the 640 h of fishway trap operation. Thus, the hourly rate of fish passage over the weir was about eight times as great as through the fishways. Most fish swam over the weir at one of its ends where water velocity was lower than at the centre (Table 1). During the 24 h of weir observation, 36 fish (or 17%) failed in their attempts to swim over the weir. Most of the unsuccessful attempts occurred near the centre of the weir. The high rate of fish passage (17 fish·h^{-1}) indicates that, when not blocked, the spillway was easily traversed by fish under the high tailwater conditions during the study (Table 3).

Data from May 12 to June 3 (when traps were checked every 3 h) revealed a diel cycle in numbers of large fish ascending the fishways. For the vertical slot and 10% Denil fishways (the ones fish used most often), frequency of ascent was lowest in the dark (00:00–06:00) and highest in the evening (18:00–24:00) (Fig. 3). Over the entire study period, spottail shiner ascended four times as frequently and large fish twice as frequently between 09:00 and 21:00 than between 21:00 and 09:00. Burbot were the only fish that ascended more frequently between 21:00 and 09:00 than vice versa (Table 4).

Pike ascended the Denil fishways and longnose and white suckers ascended the vertical slot fishway in numbers significantly higher than those expected if the ascending fish were proportioned equally among the three fishways (Table 5). Most of the longnose sucker in Table 2 were caught in the vertical slot trap before May 21 and therefore are omitted from Table 5. Pike and longnose sucker ascended all fishways with fairly uniform frequency throughout the monitoring period. However, 68 of 111 white sucker that ascended the vertical slot fishway

TABLE 2. Total numbers and fork lengths of fish caught in the fishway traps during the monitoring period (May 12 – June 25). Includes times when all three traps were not operating simultaneously and when traps were damaged by beavers. *Spottail shiner and possibly also trout-perch and juvenile yellow perch ascended these fishways but passage could not be monitored. *n* is the number of fish measured for fork length.

	Number caught			Fork length (cm)		
	Vertical slot	10% Denil	20% Denil	*n*	Mean	SD
Longnose sucker	174	16	7	26	43.5	3.2
White sucker	130	41	9	132	42.6	4.1
Lake whitefish	7	5	3	4	40.8	4.6
Northern pike	30	88	100	97	44.1	10.4
Burbot	10	17	20	26	57.4	9.2
Adult Perch	4	1	5	9	22.7	5.5
Juvenile Perch	166	*	*	166	6.5	2.1
Trout-perch	28	*	*	Approx. 3–6 cm		
Cisco	1	2	1	Approx. 18 cm		
Walleye	0	0	1	1	42.3	
Spottail shiner	77 460	*	*	220	6.9	0.96
Total (large fish)	356	170	146			

TABLE 3. Comparison of the number of fish that swam over the weir, through the spillway, and through the fishways. The 640 h of fishway monitoring were times when all three traps were operating simultaneously. The number of fish passing over the weir is not a complete count because often the entire weir could not be observed.

Location of fish passage	Hours monitored	Sucker	Pike	Unknown or others	Total
Over weir	24	63	52	63	178
Spillway	6.5	86	27	0	113
Fishways	640	203	199	45	447

and 27 of 38 that ascended the 10% Denil were caught during a single 12-h period on June 16. These fish may simply have "followed the leader," as it were, into the fishway. Nevertheless, if the June 16 fish are excluded, the data still show that white sucker had a statistically significant (G test, $P < 0.01$) preference for the vertical slot fishway. The numbers of burbot trapped in each fishway were not significantly different from each other (Table 5).

The species composition of fish caught by seining differed considerably between the downstream bay and the fishway entrance. As a proportion of the total catch (Table 6), pike were about equally represented at both locations, while the two sucker species, burbot, and whitefish were much more common near the fishway entrance (data not shown). Twenty-five of the 37 walleye and all but 1 of the 76 goldeye were caught at the downstream bay. Thirty-six walleye and one goldeye were also caught by seining upstream of the weir although seining here was usually very unproductive.

Pike and white sucker, which were the dominant large species in the seine hauls, were also the species caught most frequently in the fishway traps after June 4 (Table 6). However, the numbers of whitefish, walleye, and especially goldeye found in the traps were much lower than predicted from their representation in the seining catch. Pike, white sucker, and burbot ascended the fishways in the same ratios as they were caught by seining (Table 6).

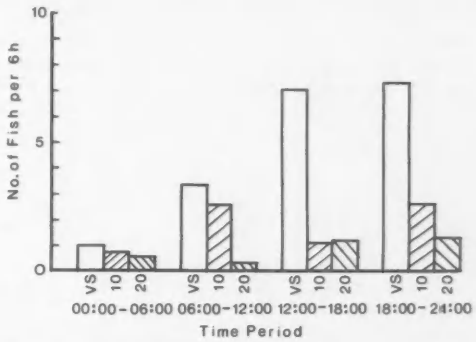

FIG. 3. Average numbers of large fish (all species except shiner, juvenile perch, and trout-perch) trapped in the three fishways (VS, vertical slot; 10, 10% Denil; 20, 20% Denil) during four 6-h portions of the day. Data were obtained between May 12 and June 3. The numbers of fish caught in the vertical slot and 10% Denil (but not the 20% Denil) were significantly (G test, $P < 0.01$) different from those expected if fish ascended with constant frequency throughout the day.

TABLE 4. Numbers of fish caught in the fishway traps during two times of day. Pike, white sucker, burbot, and other large species were caught between May 18 and June 25 when all three traps were operated simultaneously. Shiner were caught only in the vertical slot trap between May 22 and June 22. Between 09:00 and 21:00, traps were operated for 346 h for large fish and 305.5 h for shiner. Between 21:00 and 09:00, traps were operated for 294 h for large fish and 310.5 h for shiner. Catch rate per hour is shown in parentheses. Significant (G test, $P < 0.01$) differences between the two time periods are denoted by an asterisk.

	09:00–21:00	21:00–09:00
Spottail shiner*	61 360 (201)	16 100 (52)
Pike*	138 (0.40)	61 (0.21)
White sucker*	132 (0.38)	22 (0.07)
Burbot*	8 (0.02)	26 (0.09)
Other large species	35 (0.10)	25 (0.09)
All large fish*	313 (0.90)	134 (0.46)

Stomach pumping of pike was performed on five separate occasions between May 23 and June 11. The stomachs of the 87 fish examined contained a total of 410 fish of which 94% were spottail shiner. Twenty-one pike had empty stomachs.

In early June some of the trap and seine-caught pike, white sucker, and goldeye were examined to determine whether they had delayed their spawning due to the presence of the weir. This was done either by gently pressing the ventral body surface in an attempt to expel gametes in live fish or by visual examination of the gonads in dead ones. Of 45 male and 28 female white sucker that were pressed, 9 males and 1 female expelled reproductive products. None of the 9 goldeye (2 males and 7 females) and 2 of 31 male and 3 of 16 female pike that were visually examined contained unshed mature gametes. Most of the large spottail shiner that ascended the vertical slot fishway contained ripe eggs or milt.

The lactate and glucose data of group 2 exhibited a non-

TABLE 5. Numbers of large fish that ascended each of the three fishways. Only fish caught during times when all three traps were operating simultaneously are included. The *G* test was used to compare the actual number of fish collected with the numbers expected (4th row of numbers) if the ascending fish were proportioned equally among the three fishways. Significant ($P < 0.01$) differences between the three fishways are denoted by an asterisk.

Fishway	Pike	Longnose sucker	White sucker	Burbot
Vertical slot	28	29	111	5
10% Denil	80	13	38	15
20% Denil	91	7	5	14
Expected number	66	16	51	11
G value	38.4*	15.5*	125*	6.17

TABLE 6. Comparison of species composition downstream of the weir with that of fish caught in the traps. Columns A and B show the number of fish expected in the fishway traps if the species composition here were the same as in the downstream seine hauls. In column A all species are compared, while in column B only the relative proportions of pike, white sucker, and burbot are compared. Significant (*G* test, $P < 0.01$) differences between actual and expected species compositions are denoted by an asterisk.

	No. caught in traps after June 4	Expected no.		No. caught by seining (recaptures excluded)
		A	B	
Pike	163	139.3	178.2	301
White sucker	137	97.2	124.3	210
Burbot	25	17.6	22.5	38
Longnose sucker	3	7.4		16
Whitefish	2	15.7		34
Cisco	0	1.4		3
Walleye	1	17.1		37
Goldeye	0	35.2		76
G value		143.5*	2.9	

normal distribution and therefore individual values and medians are given for all data (Fig. 4). Plasma glucose and lactate were significantly ($P < 0.05$) correlated for both group 1 ($r^2 = 0.50$) and group 2 ($r^2 = 0.29$) pike. Large variations in glucose and lactate levels characterized both groups of pike. As a result, the lactate levels of pike that ascended a Denil fishway (group 1) were not significantly higher than those of group 2 which were intended to show how high lactate levels were elevated by the sampling procedure. Rather unexpectedly, plasma glucose level was significantly higher for group 2 than for group 1 pike (Fig. 4).

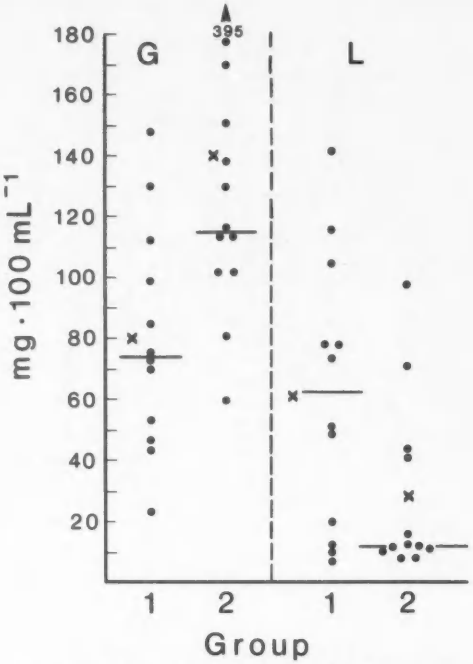

FIG. 4. Plasma glucose (G) and lactate (L) levels of pike that had volitionally ascended one of the Denil fishways in the previous 3 h (group 1) or had rested in a Denil trap for 12 h (group 2). Each dot represents the lactate or glucose value of an individual fish; median values are shown by the horizontal lines and means are marked ×. According to the Mann–Whitney *U* test, differences between groups 1 and 2 are significant ($P < 0.05$) for plasma glucose but not for lactate.

Discussion

This study provides a preliminary assessment of how effectively different fishway designs allow passage of several north-temperate, nonsalmonid fish. For efficient fish passage, water velocities within a fishway must be less than the maximum swimming speed of fish, and the distance fish must swim against a given water velocity must not exceed their endurance. A wide variety and size range of fish ascended the fishways during the study (Table 2). Therefore, the water flow regimes in Table 1 should provide a good starting point for future studies designed to identify optimal water flows for nonsalmonid fishways. Even very small fish such as spottail shiners (and possibly juvenile perch and trout-perch) could ascend the Denil fishways which were actually intended for much larger fish. We often observed shiner to rest in small areas of quiet water behind the baffles before continuing upstream and this is probably how they managed to ascend the Denils.

Although the exact water velocity against which fish swim in ascending the Denils is unknown, these fishways were much more difficult to ascend than the vertical slot. Even in the horizontal flume of the Denils fish had higher water velocities and greater distances of high velocity flow to swim through than in the vertical slot fishway. The vertical slot fishway was probably easily surmountable by virtually all sizes and species of fish in the river because many north-temperate fish can swim for several minutes against the median water velocity ($0.6\ m \cdot s^{-1}$) through the vertical slots (Beamish 1978; Jones et al. 1974).

Our data suggest that in ascending the fishways, pike preferred the Denils over the vertical slot fishway, while white and longnose sucker preferred the vertical slot over the Denils (Table 5). However, some caution must be exercised in interpreting these results. First, fish that ascended the fishways, because they probably comprised less than 10% of all upstream moving fish, may have been ones that were physically unable to surmount the weir. Therefore, the preferences shown by fish that ascended the fishways may have been different from those of fish that traversed the weir. Second, our results may not be

applicable when fish are crowded inside a fishway. When crowded, fish may simply choose the fishway that affords them the most room.

If the apparent preferences of pike and sucker for different fishways are real, these preferences may be related to the velocity of attractant water issuing from the fishway entrances. This idea is supported by Weaver's (1963) studies which showed that when given a choice of two water velocities between 0.6 and 2.5 $m \cdot s^{-1}$, salmonids choose the fishway with the higher velocity. Pike possess a large amount of anaerobic white muscle and an elongate, tubular body which allows them to swim at very high, though unsustainable, speeds (Webb and Skadsen 1980). Thus, by being predisposed to sprint swimming, pike may have preferred to swim against the higher velocity flows in the Denil fishways. Sucker do not appear to have the sprinting abilities of pike or the aerobic work capacity of salmonids. Hence, they may have found the higher velocities of the Denil fishways too challenging and therefore ascended the vertical slot fishway instead.

The possibility that fish have preferences for certain fishways has important implications. It means that the optimum fishway design may vary depending on the species it is intended to serve. Several different fishway designs may be required when efficient passage of a variety of species must be accomplished. It may also be possible to exclude undesirable species from upstream areas by building fishways that exploit interspecies differences in fishway preferences.

Attraction to high velocity flows may also explain why pike preferred to ascend the weir rather than the fishways. However, an apparent preference for the vertical slot fishways over the Denils suggests that sucker were not attracted to high velocity flows. Therefore, something other than water velocity may have caused sucker to ascend the weir in preference to the fishways. Perhaps sucker ascended the weir because there they could most easily assess both the water velocity and swimming distance they would have to surmount. Alternatively, sucker (and other fish) may have preferred the weir and spillway because they felt vulnerable or insecure in the confined space of the fishways.

Walleye and goldeye were probably traveling through the river during our study because anglers did not catch these fish at the fishway site until June 6. Yet, with one exception, walleye and goldeye did not use the fishways. That these species were seined far less frequently from beside the fishway than from the downstream bay suggests they were not attracted to the fishways, perhaps because they preferred the higher velocity flows over the weir. It is also possible that these fish were not motivated to travel upstream of the weir. Walleye are present in Lesser Slave Lake and may have been moving downstream. Goldeye are known to migrate upstream during the spring (Paterson 1966; Munson 1977), but the fishway site may be the farthest point they reach because goldeye are not known to inhabit Lesser Slave Lake. Failure to see goldeye swim over the weir (their unique body shape should have distinguished them from pike and sucker) suggests they were not motivated to travel upstream any further. It is unlikely that walleye and goldeye found the water velocities within the fishways unsurpassable because these fish have critical swimming speeds that equal or exceed those of sucker, pike, and burbot (Jones et al. 1974).

Overhead cover may be another important aspect of fishway design because it could influence the diel pattern of fishway ascent. The rarity with which our pike and sucker ascended during darkness may reflect the presence of overhead decking which could have given sufficient cover for fish to feel secure enough to ascend during the day. Nelson (1983) found that during their spring migration at Steele Lake, Alberta, pike ascended a step and pool fishway (which lacked overhead cover) as frequently at night as during daylight.

Most of the large spottail shiner contained unshed gametes and may have been on a spawning migration. However, the pike and sucker that traversed the weir and fishways during our study were probably moving back to Lesser Slave Lake after having spawned in the river. The fishways were also ascended by three species (burbot, whitefish, and cisco) that do not spawn in the spring (Scott and Crossman 1973), and by immature yellow perch. That fish ascended the fishways at times other than their spawning season and that many boreal rivers contain both spring and fall spawners indicates that fishways may be required throughout the entire open-water period.

If the pike that gather below many barriers in Alberta waterways are there solely to feed and are not on a seasonal migration, then fishways for this species may be unnecessary. However, pike continued to traverse the weir and fishways in large numbers even though they were feeding heavily and had finished spawning. Also, pike were not underrepresented in the catch from the fishway traps compared with their abundance in the seine hauls (Table 6). This indicates that pike were as motivated as other fish in the river to travel upstream past the weir. Therefore, fishways for pike are probably necessary, even at times other than the spawning season.

In previous studies (Schwalme and Mackay 1985a), reasonable estimates of resting blood glucose levels of pike in nature were obtained from fish sampled immediately after being caught by angling; these levels (for whole blood) are 45 $mg \cdot 100\ mL^{-1}$ on average. Actual resting levels were probably slightly lower than this because some elevation in blood glucose may have occurred in the delay (about 12 min) between angling and blood sampling. However, plasma glucose levels are about 4/3 of the whole blood level because intracellular glucose levels are negligible (Newsholme and Start 1973) and normal hematocrit in pike is about 25%. Therefore, resting plasma glucose levels of pike in nature (during summer) are probably about 50 $mg \cdot 100\ mL^{-1}$. Following angling, plasma glucose of pike rises to about 150 $mg \cdot 100\ mL^{-1}$ and remains elevated for at least 6 h (Schwalme and Mackay 1985a). Experiments with pike in the laboratory showed that by 30 min after exercise, plasma glucose had reached 75% of maximum postexercise levels (Schwalme and Mackay 1985b). Lactate levels in plasma and whole blood are similar and in pike, following exercise, these levels rise from 6 to 15 $mg \cdot 100\ mL^{-1}$ to about 130 $mg \cdot 100\ mL^{-1}$ and remain elevated for more than 3 h (Schwalme and Mackay 1985a, 1985b). Great elevations in plasma lactate levels are evident 5 min after exercise.

Unless all group 1 fish were sampled within 5 or 10 min after ascending the fishways, low plasma lactate levels must mean that the fish did not perform strenuous anaerobic exercise. Therefore, problems with interpretation of the biochemical data arise only when the plasma glucose and lactate levels are very high. Then one does not know if the exercise responsible for the elevated levels occurred during the fishway ascent, during blood sampling, or during attempts by the fish to escape the trap. However, pike that ascended a Denil fishway had levels of plasma glucose (median and mean were 74 and 80 $mg \cdot 100\ mL^{-1}$ resp.) and lactate (median and mean were 62 $mg \cdot 100\ mL^{-1}$) that, although higher than resting levels, were much below levels reached after angling stress. Therefore, regardless of how the elevated glucose and lactate levels were caused, the conclusion

is basically the same: ascent of the Denil fishways was at most only moderately stressful for pike compared with angling stress.

The elevation in plasma glucose level of group 2 may have been caused by some stress associated with prolonged entrapment. Therefore, the elevation above resting in the glucose levels of group 1 may have been due partly to "captivity stress" rather than to exercise. Nevertheless, elevated plasma lactate levels indicate that group 1 fish performed exercise, and because glucose and lactate levels are positively correlated (Results), exercise was probably also the major cause of the elevated glucose levels of group 1. The sampling procedure did influence plasma lactate levels because these were elevated above resting in some of the group 2 fish. Therefore, the extent of blood lactate elevation caused by fishway ascent may have been even lower than indicated by the lactate values of group 1 fish.

In summary, the vertical slot and Denil fishways, when operated under the water velocities reported here, appear capable of passing a wide variety and size range of nonsalmonid fishes. However, for optimal performance, fishways may need to be designed to suit the particular species of interest. A combination of several different fishway designs may be required when the most efficient passage of a variety of species must be accomplished.

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Distinguishing Between One-Day-Old Cod (*Gadus morhua*) and Haddock (*Melanogrammus aeglefinus*) Eggs by Gas Chromatography and SIMCA Pattern Recognition

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Knutsen, H., E. Moksnes, and N. B. Vogt. 1985. Distinguishing between one-day-old cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) eggs by gas chromatography and SIMCA pattern recognition. Can. J. Fish. Aquat. Sci. 42: 1823–1826.

Single cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) eggs were acid hydrolized. All the fatty acids were liberated and converted to methyl ester in a one-step reaction. The nonpolar hexane extract was analyzed by high resolution gas chromatography (HRGC). Selected peaks, occurring in both types of samples and in all chromatograms, were used to create a matrix for multivariate analysis. We conclude that cod and haddock eggs can be distinguished using this method.

Des œufs de morue (*Gadus morhua*) et d'aiglefin (*Melanogrammus aeglefinus*) ont été hydrolysés par l'acide. Tous les acides gras ont été libérés et transformés en esters de méthyle au cours d'une réaction ne comportant qu'une étape. On a analysé l'extrait à l'hexane non polaire par chromatographie en phase gazeuse de haute résolution (CGHR). On a utilisé certains pics, qu'on retrouvait dans les deux types d'échantillons et dans tous les chromatogrammes, pour créer une matrice permettant l'analyse de plusieurs variables. Nous concluons que les œufs de morue et d'aiglefin peuvent être differenciés à l'aide de cette méthode.

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The problem of distinguishing early stages of eggs is useful for quality control and for the estimation of the number of different species in a spawning area. Cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) spawn in the same areas and it is difficult to distinguish between the eggs. Traditional methods have shortcomings. Egg diameters overlap and the early stages, such as 1-d-old eggs, do not have any morphological differences. Biochemical approaches have been used (Mork et al. 1983), and differences in immunochemical properties (Johnson et al. 1975) have shown that there are differences between species, and also within species, with respect to enzyme composition and immunochemical properties of eggs.

The methods applied may be classified as traditional univariate chemotaxonomic methods. The use of biochemical techniques often demands a high level of skill and technique. This problem may be circumvented, however, by applying analysis of simpler compounds. Fatty acids from fish may be analyzed by gas chromatography (Henderson et al. 1984), and with simple methylation and preservation procedures contamination and misinterpretation of results is improbable. Combining high resolution gas chromatographic (HRGC) and data analytical methods capable of handling extensive data, a new approach to classification is made possible. *Multivariate chemotaxonomy* offers the possibilities of speed and certainty necessary for application in biological and related sciences. Our objective was to distinguish between eggs of cod and haddock using a combined gas chromatographic and SIMCA pattern recognition approach.

Materials and Methods

Newly fertilized cod and haddock eggs were collected from spawning basins and checked for age by microscopy. All eggs were in the cell dividing stage, i.e. 1 d old. The eggs were collected from spawning basins where several individuals were spawning. No check was made to assure that the eggs were from different individuals, although this is most likely. The average wet weight for the two groups was 1.11 mg for cod and 1.24 mg for haddock. Eggs were collected in liberal amounts of seawater by pipetting from the egg collectors. The seawater was carefully filtered on 0.45-μm GF/C filters, with a slight vacuum. Eight eggs from each type were rinsed quickly in distilled methylene chloride to remove water and adsorbed organic matter, and then *one* egg was transferred to *each* test tube. We added 1.5 mL of anhydrous 2 mol HCl/L in methanol and ensured that the eggs

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were in the solvent. The tubes were heated to 90°C for 12 h. After cooling, the methanol solution was extracted with two 1-mL portions of hexane directly in the methylation tube. We took care not to include any of the acidic methanol with the hexane. The hexane was evaporated to 40 μL with a slow constant stream of pure (grade 3, <10 ppm O_2) nitrogen gas at 40°C. A HP 7670A autosampler was used to inject 2.3 μL onto a 25-m SE-54 HP fused silica capillary column installed in a HP 5880 GC equipped with an electronic integrator and eluted with the conditions described in Fig. 1. From the 16 chromatograms, 37 peaks, which occurred in both sample types, were selected. Peaks that were well defined, i.e. symmetric, and above the lower limit for linear response of the flame ionization detector were selected. The integrated peak areas were used as a measure of peak intensities. Originally a matrix of 16 objects (samples) was constructed. This consisted of 2 samples run as a preliminary test and 14 parallel samples. From this a final matrix of 15 objects (8 cod egg samples and 7 haddock egg samples) described by 37 peaks (variables) was constructed and SIMCA pattern recognition performed.

Multivariate Analysis by the Method of SIMCA

SIMCA pattern recognition has been described by Wold (1976), and Wold and Sjøstrøm (1977), and Wold et al. (1983). The method has previously been applied to distinguish between species of fungi (Wold et al. 1979) and recently at Flødevigen Biological Station to distinguish between groups of sediment infauna (Vogt and Knutsen 1985) and to investigate feeding specificity of juvenile benthic lobsters (Knutsen and Vogt 1985).

SIMCA multivariate data analysis is one of several linear cluster analysis techniques; it uses the variance–covariance matrix to determine principal component positions (Massart and Kaufman 1983). It is intended to reduce dimensionality in multivariate data so that systematic variation present may be investigated. SIMCA may be applied in both the unsupervised, i.e. no a priori sample class (object grouping) knowledge, or in the supervised, i.e. knowledge of class, ways. After classes have been identified it is recommended (Albano et al. 1981) that separate class models be constructed for each class. The SIMCA method allows for inter- and intraclass calculation on sample (object) and parameter (variable) significance. Discrimination power (DP) describes the influence of each single variable in separating two class models. A value above 3 is considered significant (Albano et al. 1981). The discrimination power may then be used to select those variables that most clearly show class difference. The SIMCA method also gives information on variable modelling power, that is, how effective a variable is in describing a class model. A value above 0.3 is significant. When comparing two classes, if more than 30% of the variables have both low discrimination power and low modelling power, or if an object is found not to belong to the assigned class, then the variables and objects must be deleted and new models calculated.

The information available from the SIMCA analysis may be used to simplify the multidimensional description down to a uni- or bivariate one if variables are found with large discriminating and modelling power. This may be of great use in investigating changes in biochemical and/or metabolic processes in organisms that are subject to stress, e.g. pollution. The method, therefore, in addition to giving a multivariate chemotaxonomic classification may be used to screen large amounts of data so that a simplification of analytical methods may be reached.

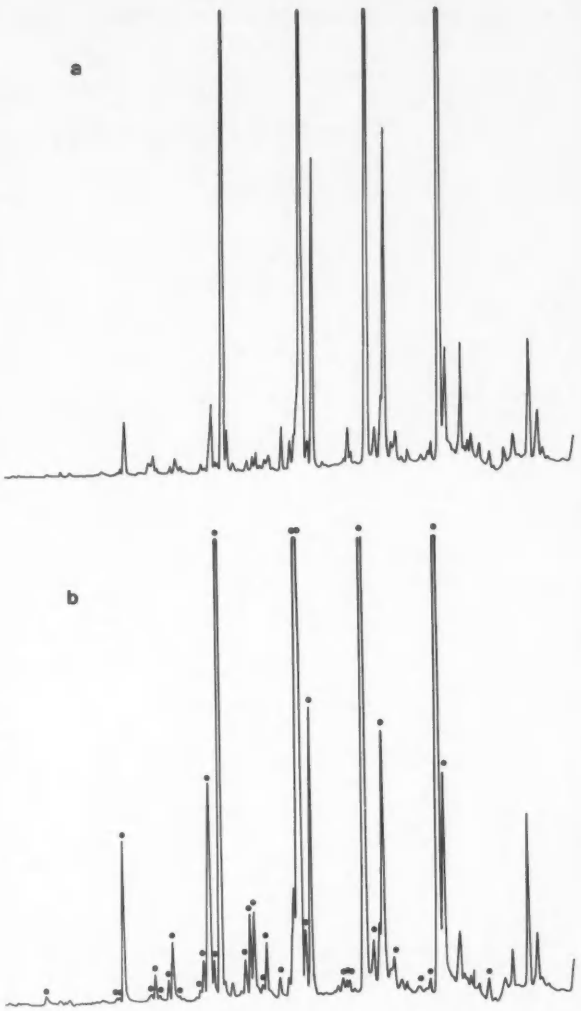

FIG. 1. Gas chromatograms of nonpolar fraction recovered from acid-hydrolyzed and methylated (a) cod and (b) haddock eggs. 2.3/40 (μL), splittless injection, splittless (90 s/250°C), detector kept at 300°C. Temperature program was 45°C (30 s), then 25°/min to 125°C (30 s), 7°/min to 275°C (10 min). Column flow: 2 mL/min of N_2 (100°C). The figure shows the part of the chromatograms between 10 and 30 min. Peaks selected for SIMCA treatment are indicated with a solid circle.

Results

The acid digestion of tissue resulted in totally dissolved material, and both bound and free fatty acids are converted to fatty acid methyl esters. Extraction twice with 1 mL of hexane gave nonpolar material approximately 1.6–1.7 mL of hexane extract. Figure 1 shows that the amount of organic material from each single egg is enough to give well-defined peaks in gas chromatographic analysis. The patterns of peaks in the chromatograms are qualitatively reproducible. Procedural blanks (not

TABLE 1. Residuals of each object to its own class and the residual standard deviation maximum (RSD-max.) of each class have been corrected for inclusion of objects in classifying class boundaries. Teta values give position of each object along respective principal components (Teta1 and Teta2) in Fig. 2. Class distance between cod and haddock eggs is 5.5.

Class 1: cod eggs 37 variables 8 objects RSD-max. (p = 0.05) = 0.8592 RSD-max. (p = 0.01) = 0.9298 Number of principal components = 2					Class 2: haddock eggs 37 variables 7 objects RSD-max. (p = 0.05) = 0.6460 RSD-max. (p = 0.01) = 0.6997 Number of principal components = 2				
Object No.	Name	Residual	Teta1	Teta2	Object No.	Name	Residual	Teta1	Teta2
01	C1	0.6310	0.51	3.80	09	H1	0.3765	5.91	2.38
02	C2	0.4154	−4.92	0.63	10	H2	0.4640	6.11	2.87
03	C3	0.7701	−4.45	0.06	11	H3	0.7428	0.17	−2.52
04	C4	0.9296	−1.56	1.94	13	H4	0.6122	3.28	−2.79
05	C5	0.6492	−4.83	1.29	14	H5	0.4238	7.40	0.34
06	C6	0.9074	−5.19	0.02	15	H6	0.4856	0.27	−6.52
07	C7	0.7322	−4.12	1.75	16	H7	0.4995	0.98	−6.43
08	C8	0.3939	0.45	3.17					

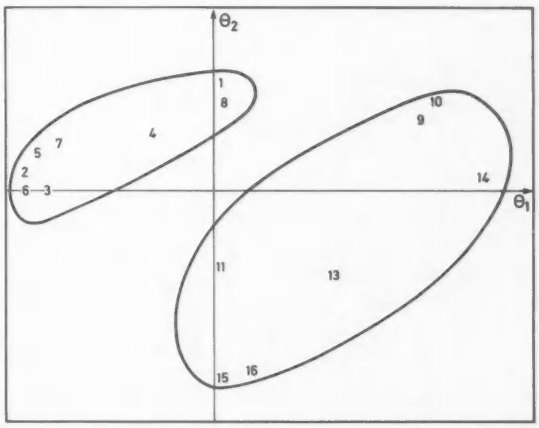

FIG. 2. Principal component plot of cod (1–8) and haddock (9–16) eggs. Thirty-seven peaks (variables) have been used to classify the 15 objects.

illustrated) show only minor contamination (1/100 to 1/1000 of sample peak intensity) at or near retention times indicated to be esters of C_{16} and C_{18} fatty acids. Qualitative standards run together with the fish egg samples indicated that the peaks mostly represented fatty acid methyl esters ranging from 14:0 to 22:6.[3] The extraction using nonpolar hydrocarbon hexane predicates that the analyzed material is most probably fatty acid esters. Further, the chromatograms in Fig. 1 show that the gas chromatogramic patterns for eggs from the two species are very similar. SIMCA pattern recognition was used to obtain principal component plots of the fish egg groups.

The method clearly shows (Fig. 2; Table 1) that the cod and haddock eggs are classified as separate groups. The class distance is 5.5. This is significant above the value of 3.0 necessary for class separation. All the objects (samples) fall within the 99% confidence interval of the class they were assigned to. The chemical analysis thus seems simple, reproducible, and has little room for random errors.

[3]Shorthand notation for carbon chain length and number of unsaturated bond(s).

Discussion

The chemical method applied is simple and rapid. Laboratory and field investigators need only test tubes and one reagent for both preservation and if necessary later hydrolysis of samples. The 2 mol HCl/L in anhydrous methanol gives a solution that acts immediately, even in the cold, as its own fixing agent. The analytical conditions applied were not optimal. Visual inspection indicates that with the peaks selected it should be possible to halve the GC analysis time to 20 min. By close inspection one might be able to distinguish selected peaks that either occur in only one of the sample types or show differences in amount between the two species. The differences are, however, subtle. As the intention of our investigation was to examine multivariate chemotaxonomic classification, we made no attempt to investigate this point. Because multivariate classification might be absolutely necessary in other circumstances where more complex problems are addressed (Vogt and Knutsen 1985), e.g. for difference in egg quality and egg age investigations (O. Grahl-Nielsen and T. Barnung, unpubl. results), the multivariate approach was tested first with fatty acids. This component of eggs was expected to be the most difficult, as the fatty acids analyzed are the least specific. Use of simple methods for analysis of amino acids and/or proteins, or unhydrolized carbohydrate material, in combination with multivariate analysis would most likely give more information, as these compounds in complex groups, e.g. peptides, are more metabolically specific. The potential for use in biochemical investigations is large. The method separates 1-d-old cod and haddock eggs into two groups and also gives information on which peaks (compounds) show the largest difference. The one haddock sample chromatogram clearly was different from the others, so that a

classification showing it to be an outlier was not a surprise. By refining (polishing) the matrices for those peaks that show significant class separation and modelling, it should also be possible to reduce the number of variables and in this way make the approach more effective for use in field investigations. The SIMCA program, where available, is easy to use and supplies classification of analyzed data within minutes. This is substantially faster than other methods.

Application of the method in combination with quantitative investigations may be useful when comparing pelagic eggs in stock estimation and for egg quality control in aquaculture rearing situations.

The use of very small sample amounts and the direct extraction combined with a qualitative comparison using pattern recognition allow the method to be applied in laboratories where advanced instrumentation is not available or is difficult to use, e.g. onboard ship.

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Seasonal Patterns in Lipid Content of Lake Michigan Macroinvertebrates[1]

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Gardner, W. S., T. F. Nalepa, W. A. Frez, E. A. Cichocki, and P. F. Landrum. 1985. Seasonal patterns in lipid content of Lake Michigan macroinvertebrates. Can. J. Fish. Aquat. Sci. 42: 1827–1832.

Lipids in several taxa of macroinvertebrates from Lake Michigan were determined seasonally to help define the role of these animals in transferring energy in the lake. Ranges of mean lipid content relative to ash-free dry weight (AFDW) throughout the year were as follows: amphipods (*Pontoporeia hoyi*), 21–54%; lumbriculid oligochaetes (*Stylodrilus heringianus*), 12–19%; tubificid oligochaetes, 9–22%; chironomid larvae, 9–39%; and *Mysis relicta*, 26–37%. A pattern of increasing lipid content during spring was observed for *P. hoyi*, chironomids, and *M. relicta*, but seasonal changes were not apparent for oligochaetes. Excluding *M. relicta*, *P. hoyi* accounted for about 65% of the biomass and 70% of the energy contained in southern Lake Michigan benthic macroinvertebrates and may assimilate up to 30% of the total bioavailable organic detritus settling into the offshore hypolimnion. Calculation of the predation loss rate (production rate minus nonpredatory death rate) of *P. hoyi* indicated that about 13 000 cal·m^{-2} (2.1 g AFDW·m^{-2}) of this amphipod may be consumed annually by fish and other predators in southern Lake Michigan.

On a déterminé sur une base saisonnière la teneur en lipides chez plusieurs taxons de macroinvertébrés présents dans le lac Michigan pour mieux définir le rôle joué par ces animaux dans le processus de transfert d'énergie dans le lac. La teneur moyenne en lipides par rapport au poids sec sans cendre (PSSC) a varié pour toute l'année de 21 à 45 % chez l'amphipode *Pontoporeia hoyi*, de 12 à 19 % chez l'oligochète *Stylodrilus heringianus* (lumbriculidés), de 9 à 22 % chez les oligochètes de la famille des tubificidés, de 9 à 39 % chez les larves de chironomidés et de 26 à 37 % chez *Mysis relicta*. On a observé en ce qui concerne *P. hoyi*, les chironomidés et *M. relicta* une augmentation de la teneur en lipides au printemps, mais il n'y a pas eu de changements saisonniers apparents chez les oligochètes. Mis à part *M. relicta*, *P. hoyi* représentait environ 65 % de la biomasse et 70 % de l'énergie contenue chez les macroinvertébrés benthiques présents dans le secteur sud du lac Michigan et il peut assimiler jusqu'à 30 % des déchets organiques utilisables qui sont produits dans l'hypolimnion au large. Le calcul du taux de pertes de *P. hoyi* par prédation (taux de production moins taux de mortalité non attribuable à la prédation) a montré que les poissons et les autres prédateurs présents dans la partie sud du lac Michigan peuvent consommer cet amphipode au rythme d'environ 13 000 cal·m^{-2} (2,1 g·m^{-2} PSSC) par année.

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Benthic invertebrates in aquatic ecosystems convert energy and nutrients from sediment detritus, microbes, and other small prey into animal biomass that is available to fish (Wells 1980) and other predators (e.g. *Mysis relicta* in the Great Lakes; Parker 1980). Lipids can be a major reservoir of biochemically stored energy. For example, lipids constituted more than one half of the total energy content of the amphipod *Pontoporeia affinis* (= *P. hoyi*) in Cayuga Lake, New York (Green 1971). Measurement of total lipid content in aquatic invertebrates is needed to quantify this reservoir of potential energy and to determine how factors such as interspecies variation (Lee 1975), organism life cycle (Tessier and Goulden 1982; Tessier et al. 1983), food supply (Goulden and Hornig 1980; Holm and Shipiro 1984), and stress (Tessier and Goulden 1982) affect lipid content. Knowledge of lipid levels in invertebrates is also needed to understand the transfer and partitioning of lipid-soluble contaminants in aquatic systems (Kenaga and Goring 1980; Scura and Theilacker 1977). Despite the importance of lipids in aquatic environments, there is only very limited quantitative data (Green 1971; Herbes and Allen 1983) on the lipid content of natural populations of freshwater invertebrates. Except for a few data on *P. hoyi*, *M. relicta*, and tubificids, collected as part of organic contaminant studies (Whittle and Fitzsimons 1983; Oliver 1984), almost no direct information is available on concentrations and seasonal trends of total lipids in Great Lakes macroinvertebrates.

Benthic amphipods, oligochaetes, and chironomid larvae, and the vertically migrating invertebrate *M. relicta*, constitute an important part of the food web in the Great Lakes. These animals affect both energy (Johnson and Brinkhurst 1971) and nutrient (Gardner et al. 1981; Gardner et al. 1983; Quigley and Robbins 1984) transformations. Other than one important study using bomb calorimetry (Johnson and Brinkhurst 1971), there have been no quantitative estimates of the rates of energy conversion and transfer by benthic invertebrates in the Great Lakes.

Our objectives were to (1) present temporal data on the lipid and estimated energy content of some common macroinverte-

[1]GLERL Contribution No. 445.

TABLE 1. Characteristics of benthos sampling sites in southeastern Lake Michigan.

Sampling depth (m)	Sediment type	Major specie of macroinvertebrates (estimated density of individuals·m^{-2})
11	Fine sand	Tubificids (7000)
29	Medium sand	*P. hoyi* (6100) *S. heringianus* (5800)
45	Silt	*P. hoyi* (10 400) *S. heringianus* (2400) Tubificids (2200)
56	Silt (high deposition area)	*P. hoyi* (7000) Tubificids (3700)

brate taxa occurring in southeastern Lake Michigan, (2) combine these data with benthos abundance data to calculate energy content per square metre for the major taxa of benthic macroinvertebrates, and (3) estimate energy flow through *P. hoyi*, the dominant benthic macroinvertebrate (Nalepa et al. 1985; Alley and Mozley 1975), in southern Lake Michigan.

Materials and Methods

Benthic Invertebrates

Invertebrates for lipid analysis were collected with a Ponar grab from four stations representing different types of sediments in southeastern Lake Michigan between March and December 1984 (Table 1). Three sites (11–45 m water depth) were located offshore from Grand Haven, Michigan, and one site (56 m), sampled only in May, was located offshore from South Haven, Michigan. Sediment samples containing the animals were placed over ice, transferred to the laboratory, and stored at 4°C until the animals were removed for analysis within 5 d. Four major groups of invertebrates were picked from the sediment: the amphipod *P. hoyi*, the oligochaetes *Stylodrilus heringianus* and tubificids (likely *Limnodrilus* sp.), and chironomid larvae. Individual animals were rinsed in lake water, dried for 4 d at 44°C in tared test tubes, and stored in a desiccator at room temperature until they were weighed for lipid analysis. Gut contents of the animals were included in measurements of dry weight and lipid content, as guts were not cleared before the animals were dried.

Mysis relicta were collected from various locations in southeastern Lake Michigan approximately 1–2 h after sunset by taking vertical tows through the entire water column with a plankton tow net (1 m diameter × 3 m long) having 561-μm mesh openings. Retained animals were placed in lake water at 4°C, supplied with a zooplankton assemblage as a food source, and returned to the laboratory for lipid analysis.

Lipid Analysis

Total lipids, relative to dry weight, were determined gravimetrically on individual animals using a microprocedure (Gardner et al. 1985) adapted from the total-lipid procedure of Folch et al. (1957). Each dried animal was extracted by homogenization in a small test tube with 100 μL of cold chloroform–methanol (2:1). The extract was separated from the residue by capillary centrifugation, and a measured portion was purified with aqueous salt solution. Lipids were quantified gravimetrically on a Cahn electrobalance after solvent evaporation. Ash-free dry weight (AFDW) of the animals was calculated by multiplying dry weights by factors of 0.87 (SE = 0.02) for *P. hoyi*, 0.92 (SE = 0.01) for *M. relicta* (unpublished data for animals from the same sampling sites and dates), and 0.90 for the oligochaetes and chironomids (Johnson and Brinkhurst 1971). The ratio of AFDW to dry weight changed slightly with season for *P. hoyi* (0.82 in April to 0.89 in June) and *M. relicta* (0.91 in April to 0.93 in June), but constant factors (mean of all measurements for each species) were used so that data treatments would be consistent among the different species.

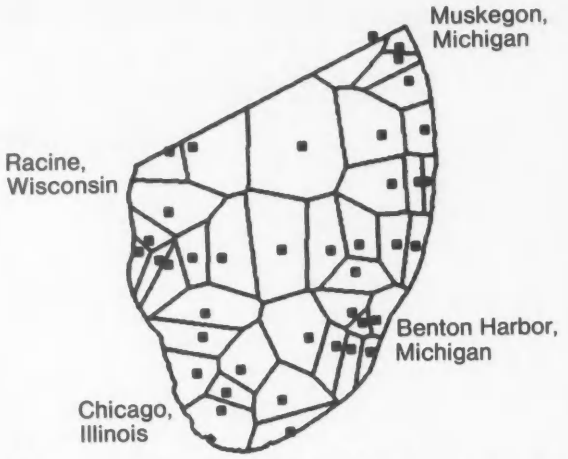

FIG. 1. Sampling stations and their representative Theissen polygon regions for calculation of mean biomass per square metre of major species of macroinvertebrates in southern Lake Michigan. Data were collected in 1980 (Nalepa et al. 1985).

Energy Content of Macrobenthos in Southern Lake Michigan

The total energy content of each benthic taxon was derived from estimates of biomass (AFDW) and lipid content. Biomass estimates were obtained from a 1980 benthic survey. Benthic samples were taken in triplicate with a Ponar grab at 40 stations (Fig. 1) in May–June, July, and September 1980. The samples were washed through a nitex screen (0.5-mm openings) and the residue was preserved in 10% formalin. Organisms were picked, counted, and separated into major taxonomic groups (amphipods, oligochaetes, chironomids, sphaeriids, and others). Biomass estimates (AFDW) were derived from length–weight relationships. For *P. hoyi*, this relationship was obtained from freshly killed individuals collected from representative stations throughout the southern end of the lake. Individuals were measured, dried at 60°C for 48 h, and ashed at 550°C for 1 h (Nalepa et al. 1985). Total areal biomass for each taxon was estimated by sectioning the sampling area into various regions best represented by different sampling sites (Fig. 1) using the Thiessen weighting method and estimating the total biomass in each region from the benthic survey. The Thiessen weighting method consists of determining the boundary about each station that is halfway between it and surrounding stations (Croley and Hartmann 1985). Species-specific AFDW from all of the regions were then summed and divided by the total lake area examined (1.30×10^{10} m^2) to obtain mean biomass per square

metre. These data were combined with mean lipid content for each taxa (averaged over all sampling sites and dates) to obtain the lipid and nonlipid energy content of benthic organisms per square metre averaged over southern Lake Michigan. Lipids were assumed to have 9.3 cal·mg^{-1}, and the remainder of the ash-free material was assumed to be protein and carbohydrate with 4.1 cal·mg^{-1} (Green 1971).

Results and Discussion

Lipids in Lake Michigan Macroinvertebrates

Total lipid levels varied among the various invertebrate taxa in Lake Michigan (Fig. 2). Mean lipid values were highest in *P. hoyi* and *M. relicta* (21–54% of AFDW). These species are important prey for small fish in the Great Lakes (Wells and Beeton 1963; Dryer et al. 1965; Wells 1980). The oligochaetes *S. heringianus* and Tubificidae had lipid levels of 9–24%, whereas Chironomidae had a wider range of 9–39% of AFDW (Fig. 2). Our values for *P. hoyi* are comparable with levels previously reported for this species (24–33% of dry weight; Green 1971; Whittle and Fitzsimons 1983). However, values for *M. relicta* are considerably higher than those previously reported for this species in Lake Ontario (Whittle and Fitzsimons 1983), possibly because of different extraction techniques. *Pontoporeia hoyi* and *M. relicta* had lipid levels within the range of those reported for cold-water marine species of Arctic zooplankton (14–64% of dry weight; Lee 1975). The values for oligochaetes and chironomids are similar to the levels previously reported for freshwater invertebrates (11–32%; Herbes and Allen 1983).

When data from all dates and sites were combined, a significant positive relationship between AFDW and percent lipid content was observed for *P. hoyi* (% lipid = 31.08 + 4.03 × AFDW; $R^2 = 0.083$, $P = 0.001$, $N = 181$). In contrast, a significant negative linear relationship was observed for *S. heringianus* (% lipid = 21.05 − 4.84 × AFDW; $R^2 = -0.212$, $P = 0.0001$, $N = 145$), Tubificidae (% lipid = 19.23 − 6.91 × AFDW; $R^2 = -0.094$, $P = 0.008$, $N = 63$), and Chironomidae (% lipid = 33.47 − 37.30 × AFDW; $R^2 = -0.135$, $P = 0.05$, $N = 29$). Although significant, these relationships accounted for only a small fraction ($R^2 < 0.25$) of the total variance in lipid content for any of these species. *Mysis relicta* did not exhibit a significant relationship between weight and lipid content ($R^2 = 0.009$, $P = 0.574$, $N = 36$).

Seasonal trends in lipid content were apparent in *P. hoyi*, Chironomidae, and, to a lesser extent, *M. relicta* (Fig. 2). Lipids in *P. hoyi* collected from 29 m increased linearly from April to June, but then decreased gradually during July through December (Fig. 2). This trend is similar to seasonal changes in growth for *P. affinis* in the Northern Baltic proper and to seasonal variations in lipid content for some marine invertebrates (Conover and Corner 1968; Morris 1971). It probably reflects seasonal changes in the quality and quantity of food supply or could be due in part to physiological changes due to reproductive and developmental changes in the animals (Tessier and Goulden 1982). Seasonal growth in *P. hoyi* is strongly dependent on food supply (Johnson and Brinkhurst 1971; Cedarwall 1977). Growth efficiencies for both young and juvenile *Pontoporeia* in Lake Ontario were high during the spring period and then decreased during the summer months (Johnson 1970). These high growth efficiencies, and our observed high lipid content, may have been caused by the high quality of food

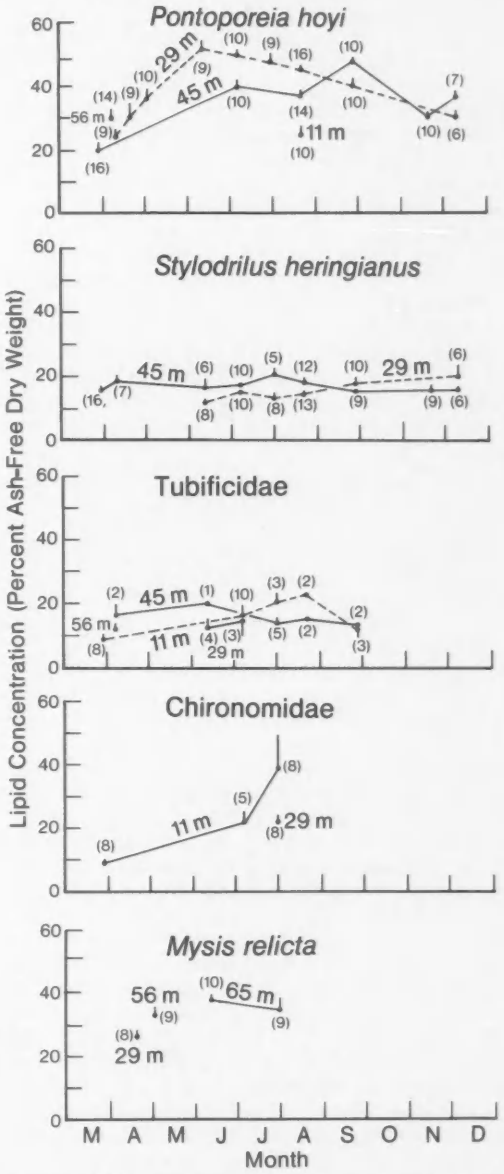

FIG. 2. Total lipid content, expressed as a percentage of ash-free dry weight, in major species of Lake Michigan macroinvertebrates sampled in southeastern Lake Michigan in 1984. Range bars = SE. Numbers in parentheses represent the number of replicate animals analyzed.

(mainly diatoms) following the spring phytoplankton blooms. Many of the spring diatoms settle directly to the sediments, where they would be available to the benthos, because pelagic zooplankton are not sufficiently abundant in spring to quantitatively graze the phytoplankton (G. Fahnenstiel and D. Scavia, NOAA Great Lakes Environmental Research Laboratory, Ann Arbor, MI). It is reasonable that lipids would accumulate after a new source of food enters the hypolimnion as a result of the spring diatom bloom because *P. hoyi* feeds mainly on detrital

material at the sediment surface (Marzolf 1965) and has the ability to store energy as triglycerides. Triglyceride storage products constituted more than 60% of the total lipids of *P. hoyi* sampled in August (Gardner et al. 1985). The decrease in lipid content observed from July to December at 29 m may have resulted from the animal's use of the lipids for energy as a result of decreased food supply. Lipid levels in *P. hoyi* at 45 m between March and August were lower than levels at 29 m during the same period, but seasonal trends in lipid content were the same for animals from both sites. However, compared with concentrations at 29 m, lipid levels at 45 m appeared to reach a maximum later in the season and showed more variations among successive samplings.

Insufficient numbers of chironomid larvae were examined to describe detailed seasonal trends, but lipids in animals sampled in July were higher than in those sampled in March (Fig. 2). *Mysis relicta* collected from a variety of stations exhibited trends in lipid content similar to *P. hoyi*, but changes were not as pronounced (Fig. 2).

Consistent seasonal trends were not apparent for either *S. heringianus* or Tubificidae. These animals apparently do not accumulate a great deal of lipids as an energy reserve; their mean lipid levels never exceeded 25% of AFDW. The low lipid content and general lack of seasonal trends in oligochaete lipid levels may be related to feeding habits. These differences between oligochaetes and the other macroinvertebrates support the general hypothesis that those species living in environments where food supply is unpredictable or varies greatly in abundance should store more energy than those species exposed to more uniform food supplies (Slobodkin and Richman 1961; Griffiths 1977). Oligochaetes are subsurface deposit feeders and may be exposed to a more uniform food supply during the year than are *P. hoyi* and chironomids that may feed on surface materials, or *M. relicta* that feed in the pelagic zone. In contrast with the high levels of triglyceride storage products in *P. hoyi*, *S. heringianus* sampled at the same stations in August did not have high levels of triglycerides (Gardner et al. 1985), and in addition, their lipid content did not change consistently with season (Fig. 2).

The differences in levels and seasonal trends of lipid content among the different taxa of macroinvertebrates may also have been related to differences in the lipid content of their food. For example, accumulation of fats by daphnids was directly correlated to the percentage of algal fat in their diet (Cowgill et al. 1984). Similar relationships between animal lipid content and food composition may be expected for benthic invertebrates. In agreement with this hypothesis, *P. hoyi*, *M. relicta*, and chironomids that feed on relatively "fresh" detrital material at the sediment surface or suspended in the water column had relatively high lipid contents that varied seasonally, with levels increasing in spring and summer when "high-quality" detrital inputs should be higher. If these animals ingest a large portion of the available detritus and efficiently remove much of the available lipids from this material, oligochaetes may be exposed to lower levels of lipids in their subsurface food supplies. More work is needed to define mechanisms of lipid and energy transfer from detrital material into macroinvertebrate biomass.

The variation in lipid levels among the various taxa of macroinvertebrates may be an important factor affecting the bioaccumulation of nonpolar organic contaminants by these animals in the lakes. For example, because these contaminants are highly lipophilic, the high levels of lipids in *P. hoyi* are consistent with the predicted high bioconcentration factor (BCF, that is, the steady-state ratio of contaminant concentration in an organism to its concentration in the water) for polycyclic aromatic hydrocarbons in this amphipod. This high BFC results from extremely slow depuration rates (Landrum 1982). In contrast, the depuration rate of *S. heringianus* with lower lipid levels is almost an order of magnitude faster than the rate for *P. hoyi* (Frank et al. 1985). The depuration, and therefore turnover, rates of organic contaminants may be controlled in part by the content, composition, and turnover rates of component lipids in these invertebrates (Kenaga and Goring 1980; Scura and Theilacker 1977).

TABLE 2. Comparison of seasonal and site ranges in mean energy content of Lake Michigan macroinvertebrates with ranges for similar species examined in the Bay of Quinte and Lake Ontario (Johnson and Brinkhurst 1971). Energy contents of Lake Michigan species were calculated from lipid concentrations; others were done by bomb calorimetry.

	Calories·mg AFDW^{-1}	
	Lake Michigan	Bay of Quinte and Lake Ontario
P. hoyi	5.2–6.9	5.7–7.1
S. heringianus	4.7–5.1	—
Tubificidae	4.5–5.3	5.4–5.7
Chironomidae	4.5–6.1	4.8–6.6
M. relicta	5.4–6.0	—

Energy Content of Lake Michigan Macroinvertebrates

Energy content of the various taxa was estimated to consider amounts of "potential energy" stored in these macroinvertebrates in southern Lake Michigan. Because lipids have more than twice as many calories per unit dry weight as proteins or carbohydrates, the lipid content affects the energy content of organisms relative to biomass. The mean calculated energy content in the species examined ranged from 4.5 cal·mg AFDW^{-1} for Chironomidae and Tubificidae sampled in March (9% lipid) up to 6.9 cal·mg AFDW^{-1} for *P. hoyi* sampled in June (54% lipid; Fig. 2; Table 2). Although slightly lower, the ranges of mean calculated caloric values over the different dates and sampling sites are generally similar to values determined with a bomb calorimeter for similar taxa in the Bay of Quinte and Lake Ontario (Johnson and Brinkhurst 1971; Table 2).

The total and relative energy content of these organisms in southern Lake Michigan were estimated using relative abundances in 1980 and our estimates of energy content for each benthic taxon (Table 3). Lipids were not measured in sphaeriids, but this group constitutes less than 3% of the total macrobenthic biomass in this region (Nalepa et al. 1985). *Mysis relicta* were not considered in these calculations because of their pelagic distribution and because detailed biomass data were not available.

Pontoporeia hoyi is the dominant benthic macroinvertebrate in terms of energy storage in southern Lake Michigan (Table 3). It had a mean caloric content of 16 500 cal·m^{-2} or about 70% of the total caloric content in the benthic macroinvertebrates (excluding sphaeriids). This estimate agrees with previous estimates of 13 824 – 28 274 cal·m^{-2} for offshore Lake Michigan (Lubner 1979) based on three sampling stations for biomass and reported caloric values for *P. hoyi* (Green 1971; Johnson and Brinkhurst 1971).

TABLE 3. Mean lipid concentration and areal biomass and energy content for four species of benthic invertebrates averaged over southern Lake Michigan.

Species	Mean lipid concn. (% AFDW)	Mean biomass (g AFDW·m^{-2})	Energy content (cal·m^{-2})	Species contribution to total caloric content (%)
P. hoyi	38	2.72	16 500	71
S. heringianus	16	0.83	4 100	18
Tubificids	15	0.54	2 600	11
Chironomids	23	0.026	140	<1
Total		4.12	23 300	100

Energy Flow through *P. hoyi* in Lake Michigan

We estimated minimal food requirements of *P. hoyi* and then calculated how many calories of fish could be supported by *P. hoyi*, based on *P. hoyi* production rates, to assess the importance of *P. hoyi* in making detrital energy available to predators. Making these calculations from areal biomass and caloric content data required a conversion of observed biomass to production rates. For *P. hoyi*, the annual production rate to standing crop biomass ratio has been estimated to be 1.1·yr^{-1} (Lubner 1979) and 1.27·yr^{-1} (Winnell and White 1984) for offshore sites (>40 m) and 3.13·yr^{-1} for a nearshore (15 m) site (Winnell and White 1984) in Lake Michigan. Because *P. hoyi* abundances are greater offshore, annual production was assumed to equal 1.2 times the standing stock, the mean of the offshore ratios. This ratio is similar to those previously reported for *Pontoporeia* sp. in other areas (Cederwall 1977). Based on this ratio, mean annual production of *P. hoyi* was calculated to be 3.3 g AFDW·m^{-2}·yr^{-1} (2.76 g AFDW·m^{-2} × 1.2·yr^{-1}). Assuming a mean caloric content of 6.1 cal·mg AFDW^{-1} (lipid content of 38% of AFDW; Table 3), this would represent 20 000 cal·m^{-2}·yr^{-1}.

Both the assimilation efficiency (food assimilated/food consumed) and the growth efficiency (food used for growth and reproduction/food assimilated) should be considered to calculate how much detrital food would be required to produce this biomass. Benthic animals that feed on sediments and detritus are thought to have relatively low assimilation efficiencies because they subsist mainly on low-quality food but high growth efficiencies because they expend relatively little energy in finding food (Johnson and Brinkhurst 1971). There is at present no direct information on assimilation efficiencies of *P. hoyi*, but we estimated their detrital food requirements conservatively from information on growth efficiency. Average growth efficiencies measured for *P. hoyi* and a variety of other benthic invertebrates in Lake Ontario and the Bay of Quinte ranged from 0.3 to 0.6 (Johnson and Brinkhurst 1971). If this range of growth efficiencies is assumed for Lake Michigan animals and the annual production of *P. hoyi* is 3.3 g AFDW·m^{-2}·yr^{-1}, then *P. hoyi* would have to assimilate between 5.5 and 11 g of organic detritus (32 000 – 63 000 cal·m^{-2}·yr^{-1}). These values represent 11–23% of the total influx of organic detritus (as measured by summer sediment traps at offshore stations) or between 17 and 34% of the labile material (total input minus net accumulation in the sediments) coming into the hypolimnion the same year (Eadie et al. 1984). This assumes that organic carbon constitutes one half of the total organic material (Strickland 1960) and that influx of particles during the summer is the same as the average during the rest of the year. The portion of detrital input actually ingested by *P. hoyi* would likely be much greater than these estimates because assimilation efficiencies of amphipods are generally less than 20% (Hargrave 1970; Barlocher and Kendrick 1975). Possibly, *P. hoyi* could ingest most of the fresh detritus coming into the hypolimnion.

The amount of fish biomass supported by *P. hoyi* in Lake Michigan can be calculated by estimating the predation rates. The mean natural life span of *P. hoyi* in offshore Lake Michigan is about 2.5 yr (Lubner 1979). Based on this life span, the nonpredatory death rate would be 40% of the population per year. Since predation is likely the major factor causing early mortality in most individuals, the predatory losses from the *P. hoyi* population can be estimated as the difference between the production rate (120%·yr^{-1}) and nonpredatory death rate (40%·yr^{-1}), yielding a predation rate of 80% of the standing crop per year. In terms of energy equivalents, this amounts to about 13 000 cal·m^{-2} potentially consumed by predators each year. Assuming that most predation losses in *P. hoyi* are due to small (generally <250 mm long) fish (e.g. alewife (*Alosa pseudoharengus*), bloater (*Coregonus hoyi*), yellow perch (*Perca flavescens*), spottail shiner (*Notropis hudsonius*), trout-perch (*Percopsis omiscomaycus*), slimy sculpin (*Cottus cognatus*), and fourhorn sculpin (*Myoxocephalus quadricornis*); Wells 1980; Wells and Beeton 1963), and that these fish, in turn, have an energy conversion efficiency of 10–20% (Stewart et al. 1983), predation on *P. hoyi* alone could produce 1300–2600 calories of fish·m^{-2}. Unfortunately, estimates of total annual production for fish in this size range are not available for comparison (E. Brown and L. Wells, Great Lakes Fishery Laboratory, Ann Arbor, MI, pers. comm.). Also, data were not available to make the predator-loss calculation for other benthic macroinvertebrates that we examined, but their cumulative contribution would probably be much smaller than that of *P. hoyi* because of lower biomass and areal productivity and because they are apparently less desirable as a food for many predators. *Mysis relicta* may have an impact on fish production comparable with *P. hoyi*, particularly in deeper regions of the lake. Although its areal biomass (0.11–1.11 g dr weight·m^{-2}; Sell 1982) tends to be lower than that of *P. hoyi*, its production rate (0.25–3.2 g dry weight·m^{-2}·yr^{-1}; Sell 1982) and lipid content (Fig. 2) are relatively high and it is a desirable prey for fish (Wells and Beeton 1963; Dryer et al. 1965; Wells 1980).

These calculations show that macroinvertebrates, particularly *P. hoyi*, are quantitatively important to energy transformations and food chain dynamics in Lake Michigan. These animals are a major mechanism for transferring energy from detritus and microbes entering the hypolimnion into small fish that serve as

food for larger fish in the lake. The differences in lipid levels and seasonal trends imply that the various taxa have different roles in assimilating and transferring energy and contaminants through or from the detrital food web. Macroinvertebrates that feed at the sediment surface (*P. hoyi* and chironomids) or in the pelagic zone (*M. relicta*) have higher and more seasonally variable levels of lipids than the subsurface-feeding oligochaetes. Our comparison of food requirements for *P. hoyi* with trap estimates of sedimenting detritus suggests that these animals assimilate a sizable portion of newly available detrital material entering the hypolimnion. Since approximately one half of the assimilated energy is lost through respiration (Johnson and Brinkhurst 1971), these invertebrates must also be responsible for much of the mineralization of detrital material in the hypolimnion and sediments of Lake Michigan.

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NOTES

Simplified Calculation of Optimum Spawning Stock Size from Ricker's Stock Recruitment Curve

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Hilborn, R. 1985. Simplified calculation of optimum spawning stock size from Ricker's stock recruitment curve. Can. J. Fish. Aquat. Sci. 42: 1833–1834.

The optimum spawning stock size for a Ricker stock recruitment curve was shown to be accurately approximated by the equation $P_s = P_r(0.5–0.07a)$ when $0 < a < 3$. A simple modification was also shown to incorporate stochastic variation about the stock recruitment curve into calculations of optimum stock size.

L'auteur démontre que la taille optimale d'un stock reproducteur calculée à l'aide d'une courbe de recrutement de Ricker peut être précisément établie d'après l'équation $P_s = P_r(0{,}5–0{,}07a)$ quand $0 < a < 3$. Il montre aussi qu'une simple modification inclut la variation stochastique de la courbe de recrutement du stock dans le calcul de la taille optimale d'un stock.

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The Ricker stock recruitment curve (Ricker 1954) has been widely used in population dynamics, and there are many studies that fit the Ricker curve to data and then calculate the optimum escapement. When dealing with semelparous species, such as Pacific Salmon (*Oncorhynchus* sp.), the spawning stock size that maximizes the long-term average catch (i.e. the stock size for MSY P_s in Ricker's notation) depends only on the parameters of the stock recruitment curve. The optimum spawning stock size is calculated from a transcendental equation requiring iterative calculations. While this is not a serious problem given the wide availability of programmable calculators and microcomputers, it prevents pencil-and-paper calculations of optimum escapement and is a practical impediment to many fisheries managers with little mathematical training. My objective was to show that a simple linear equation approximates the transcendental equation very accurately within the range of stock recruitment parameters appropriate to most semelparous species. I also aimed to show that although the stock recruitment parameters estimated from the linear regression method commonly used underestimate the average recruitments for any level of spawners, a simple correction for this exists and can be incorporated into the calculation of optimal stock size.

Using the notation of Ricker (1975), the stock recruitment model is

$$(1) \quad R = Pe^{a(1-P/P_r)}$$

where R is the recruits produced, P is the spawning stock size, a is a parameter of productivity so that $R = Pe^a$ when P is very small, and P_r is the unfished equilibrium stock size. The spawning stock size at MSY (P_s) is found by finding the value of P_s that solves the following equation:

$$(2) \quad 1 = (1 - aP_s/P_r)e^{a(1-P_s/P_r)}.$$

Equation (2) is normally solved for the value of P_s iteratively using a gradient search technique such as Newton's method. However, note that the ratio P_s/P_r is independent of P_r (P_r is a scaling parameter), and therefore the ratio P_s/P_r depends only on a. If one plots P_s/P_r vs. a, the curve is almost perfectly linear for a values between 0 and 3, and the line in this range can be approximated very accurately by the equation

$$(3) \quad P_s = (0.5 - 0.07a)P_r.$$

Table 1 shows the calculations for $0 < a < 5$. The coefficients of 0.5 and 0.07 are not exactly those obtained by calculating the linear regression of the data for $0 < a < 3$ from Table 1, but are extremely close and I use these for ease of recollection.

The maximum recruits per spawner occurs when stock size is close to zero and is e^a. Since $e^3 = 20.086$, the range $0 < a < 3$ is certainly useful for all semelparous fish that I know of. Thus, I have found the above approximation universally applicable for work on Pacific salmon. Some highly fecund insects and perhaps some fish may have $a > 3$.

Equation (1) is a deterministic stock recruitment curve. There are numerous alternative methods to convert this curve to a stochastic curve, depending on how one assumes natural variability to enter the stock recruitment dynamics. The most com-

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TABLE 1. Ratio between the optimal spawning stock P_s and the unfished equilibrium stock size P_r and the approximate solution using the formula $P_s/P_r = 0.5 - 0.07a$.

Ricker a value	Optimal P_s/P_r	Approximate P_s/P_r	% error
0.0	0.5000	0.500	0.00
0.5	0.4675	0.465	0.53
1.0	0.4328	0.430	0.65
1.5	0.3968	0.395	0.45
2.0	0.3607	0.360	0.19
2.5	0.3258	0.325	0.25
3.0	0.2933	0.290	1.13
3.5	0.2639	0.255	3.37
4.0	0.2381	0.220	7.60
4.5	0.2157	0.185	14.23
5.0	0.1964	0.150	23.63

monly used stochastic version is

$$(4)\quad R = Pe^{a(1-P/P_r)}e^{\epsilon}$$

where ϵ is normally distributed with a mean of 0.0 and a standard deviation of σ_ϵ. This lognormal error assumption is used for three reasons. Most importantly, it has been shown empirically (Peterman 1981) that the variability in stock and recruitment for salmon is often lognormally distributed. Second, the mathematical properties of the lognormal distribution are convenient. And finally, the traditional method of estimating the parameters a and P_r implicitly assumes a lognormal distribution.

The normal method for estimating the parameters is to rewrite equation (4) as

$$(5)\quad \ln(R/P) = a + (a/P_r)\,P + \epsilon.$$

There is some misunderstanding about biases in the estimation of equation (5). On seeing expression (5) with the ratio R/P on the left-hand side and P on the right-hand side, one might assume that equation (5) leads to biased estimates. Walters (1985) and Goodyear and Christensen (1984) discussed the biases in the estimation using this method and showed that they are due to time-correlation in the data. In fact, P is a random variable through the influence of ϵ on R, and unless the P values are deliberately chosen to destroy the correlation, a bias will result. This bias has nothing to do with using the ratio R/P.

Equation (5) is computationally convenient; it is a simple regression and is only slightly biased by the time series effect when the sample size is large (25+ points) and there is good contrast in the historical P time series (Walters 1985). However, given the assumption of lognormal errors in equation (5), we can write the "proper" nonlinear estimation equation as

$$(6)\quad \ln(R) = \ln(P) + a - (a/P_r)P + \epsilon$$

and then find the values of a and P_r that minimize the sum of ϵ squared. This also corresponds to the maximum likelihood estimate of a and P_r. Fortunately it is computationally identical to equation (5), since all we are doing is shifting ln (P) from the right to the left-hand side of the equal sign. The values of a and P_r that minimize the sum of ϵ's squared are the same.

It is equally convenient that the normal regression statistics provide us with an estimate of σ_ϵ, the residual error variance of the regression. The variability represented by σ_ϵ is important because the parameters from equation (5) estimate the Ricker curve that passes through the mode of the data at each spawning stock size. This is because the modal, most probable value of e^ϵ is $e^0 = 1$. However, the mean of e^ϵ is $e^{\sigma_\epsilon^2/2}$. Thus, the arithmetic average recruitment at any stock size will be larger than the modal recruitment (see Ricker 1975 for discussion). The arithmetic average stock recruitment curve is specified by

$$(7)\quad R = Pe^{a(1-P/P_r)+\sigma_\epsilon^2/2}.$$

We can now calculate the optimum escapement adjusted for stochastic variability P_s' as

$$(8)\quad P_s' = P_r'(0.5 - 0.07a')$$

where

$$a' = a + \frac{\sigma_\epsilon^2}{2}$$

$$P_r' = P_r a'/a.$$

To empirically validate equation (7), I generated 200 000 simulated stock recruitment points with different a and ϵ values. I found that equation (7) does represent the expected arithmetic average.

The two simple equations (3) and (8) enable one to calculate the optimum escapement much more easily than in the past and to incorporate the amount of stochastic variability in the stock recruitment curve into an analysis of optimum escapement.

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Use of Visual Census for Estimating Population Size in Northern Pike (*Esox lucius*)

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Turner, L. J., and W. C. Mackay. 1985. Use of visual census for estimating population size in northern pike (*Esox lucius*) Can. J. Fish. Aquat. Sci. 42: 1835–1840.

A stratified systematic sampling regime was used to survey numbers of northern pike (*Esox lucius*) (age 1 yr or older) on five different 2-d sampling periods during the summer months (June, July, and August) in Roi Lake, Alberta, a small, deep, meromictic lake with a simple basin morphometry and high water clarity. The final combined estimate was 180 ± 39 northern pike (23 pike/ha of lake surface). Detailed underwater observation within a specified area was used to assess depth distribution, sample independence, and reaction of northern pike to the presence of the observer. Northern pike selected depths of less than 1 m, and this distribution pattern was constant throughout the summer months, justifying the stratified methodology utilized in the population estimate. Samples were 92% independent, and the reaction of pike to a diver contributed an estimated 4% error to the accuracy of the estimate.

On s'est servi d'une technique d'échantillonnage systématique stratifiée pour recenser le nombre de grands brochets (*Esox lucius*) d'un an ou plus au cours de cinq périodes différentes d'échantillonnage de 2 jours pendant les mois d'été (juin, juillet et août) dans le lac Roi (Alberta), qui est un petit lac méromictique profond ayant la morphométrie d'un simple bassin et où l'eau est très claire. L'estimation finale combinée a donnée 180 ± 39 grands brochets (23 brochets/ha de surface du lac). On a procédé à une observation détaillée sous l'eau dans un secteur déterminé afin d'évaluer la distribution selon la profondeur, l'indépendance des échantillons et la réaction du grand brochet à la présence de l'observateur. Le grand brochet affectionnait les secteurs de moins de 1 m de profondeur et ce mode de distribution n'a pas varié pendant les mois d'été, ce qui justifie la méthode d'échantillonnage stratifiée qui a servi à estimer la population. Les échantillons étaient indépendants à 92 % et la réaction du brochet à la présence d'un plongeur a entraîné un taux d'erreur évalué à 4 % pour ce qui est de la précision de l'estimation.

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A fundamental aspect of any population study is an estimate of population size. The significance of this parameter has stimulated the development of a number of procedures, both direct and indirect, to estimate either relative or absolute population size. The most appropriate procedure depends upon the objectives of the study and characteristics of the species being studied, as well as the time, resources, and personnel available.

As part of a research program investigating the distribution and activity of northern pike (*Esox lucius*) in Roi Lake, Alberta, an attempt was made to estimate the size of the resident population using a visual census technique. The method of estimating population size was designed to determine the number of pike age 1 yr or older in Roi Lake. The depth distribution of pike within the lake was examined on a monthly basis and was used to detect any changes in distribution that would affect the efficiency of the sampling design. This report describes the technique used and the precision of the estimate obtained.

Strengths and limitations of estimation methods — Numerous methods are available for estimating fish population size (Everhart et al. 1975). We concluded that standard methods such as rotenone sampling and catch reduction per unit effort were incompatible with our planned behavioral observations. Various mark–recapture techniques were suitable with respect to their nondestructive nature; however, validation of the general assumptions of these methods (random sampling, complete mixing of the population) was not possible. Due to the differential response to catching gear by different size categories within the resident population, it would have been difficult to capture a random sample of northern pike in Roi Lake without the use of a number of complementary capture techniques. In addition, a tagging study designed to detect major distributional changes in the population of Roi Lake indicated that large-scale movements (> 100 m) by individuals were limited during the summer months (L. J. Turner, unpubl. data). Hence, the assumption of homogeneous dispersal of marked animals (complete mixing) within the entire lake between the time of release and the time of the subsequent recapture sample could not be justified.

The swimming of transects by free divers has been used to obtain approximate counts of fish numbers in both tropical and temperate waters (Brock 1954; Northcote and Wilkie 1963; Keast and Harker 1977; Werner et al. 1977; Leum and Choat 1980; Helfman 1983). The visual census technique provides a relatively rapid and efficient method of estimating population

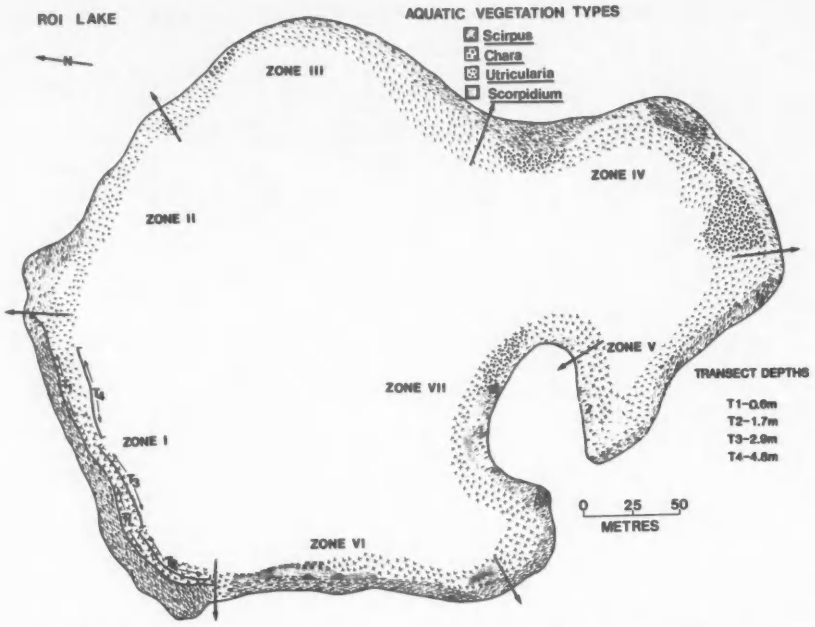

FIG. 1. Macrophyte distribution, position of transect lines, and zone demarcation in Roi Lake.

size without disturbing the fish assemblage by taking a physical sample (Northcote and Wilkie 1963; Sale and Douglas 1981).

There are, however, various assumptions and constraints involved in using the visual census technique which should be considered in the choice and design of the experimental procedures. The major constraint is its necessary confinement to reasonably clear water and moderate to shallow depths (<30 m). Thus, in many eutrophic lakes with characteristically low visibility this method is unsuitable. A major assumption of the visual census estimate is that the total number of fish observed constitutes a measure of the actual number present. The validity of this assumption depends upon various observational variables: crypticity of the fish (or the screening effect of the habitat), hiding or dispersion of alerted fish in front of divers, varying visibility of different size classes, duplicate counts, experience and speed of the observer, and conditions related to weather, turbidity, etc. In most cases, these problems are reflected in a conservative population estimate (Brock 1954; Northcote and Wilkie 1963; Hirst 1969; Emlen 1971; Keast and Harker 1977; Helfman 1983).

Constraints of the visual census technique may be overcome by selecting a suitable study site. Certain observational difficulties may be alleviated with appropriate methodology, or the error estimated by appropriate experimentation and analyses.

Certain ecological and behavioral features of northern pike make them amenable to visual census techniques. Pike are inactive greater than 80% of the time during the daylight hours of the summer months (Diana 1980; L. J. Turner, unpubl. data) and are typically located in the illuminated littoral zone of north-temperate lakes (Diana et al. 1977; Chapman and Mackay 1984a, 1984b).

Methods

Study site — Roi Lake is a small, deep (max depth = 21 m), meromictic lake located 40 km northwest of Edmonton, Alberta (53°51′N, 114°05′W) with a simple basin morphometry. The lake has a surface area of 8 ha, a manageable size for surveying since a large area can be sampled in proportion to the total area, and the sampling can be repeated. The littoral zone was composed of a nearly continuous bench of the emergent macrophyte *Scirpus* extending as far as 20 m from shore to a depth of 0.8 m, and a steep slope continuing to a depth of 5 m dominated by the macroalga *Chara* (Fig. 1). The littoral area was relatively homogeneous with respect to these vegetation characteristics. The lake was thermally stratified with the metalimnion commencing at a depth of 4–5 m. Characteristic of meromictic lakes, the hypolimnion (>8 m in depth) is permanently anoxic, thus limiting the distribution of the resident fishes to depths of less than 8 m. The unproductive nature of Roi Lake was reflected in good visibility; a mean Secchi disk reading of 6.4 ± 1.2 m (standard deviation, n = 20) was recorded during the period of investigation.

Population estimate — As a preliminary survey of the distribution of northern pike (age 1 yr or older), randomly selected areas of the lake were censused by snorkel and SCUBA in the first 2 wk of June 1982. Young-of-the-year were not included in the estimate, as mortality is extremely high during the first 4–5 mo of life, and numbers thus show marked fluctuations over time (Franklin and Smith 1963; Mann 1980). Results of this survey indicated that 90% of the observed pike were associated with vegetation and in less than 1.5 m of water.

Use of the entire lake as a discrete sampling unit would be the easiest to deal with mathematically and would provide an unbiased estimate. However, the apparent depth selection exhibited by northern pike in the preliminary survey indicated considerable spatial variation. Thus, a stratified systematic regime based on depth was considered to be the appropriate sampling method.

The lake was divided into seven zones 150–200 m in length

(Fig. 1). End boundaries of the zones were demarcated on the shoreline with surveyor's tape and underwater using bricks tagged with fluorescent tape. Zone VII was omitted from the survey to avoid disturbing nesting loons. Boundaries of the seven zones are illustrated in Fig. 1 (transects used in the examination of depth distribution, T1–T4, in zone 1 are indicated; transects used for the population estimate are not shown). Each zone was subdivided into three regions: area A, characterized by a high density of pike (<1.0 m in depth); area B, characterized by a low density of pike (1.0–3.0 m in depth); and area C, characterized in general by the absence of pike (>3 m in depth). The strip census technique was applied using areas A and B; area C was eliminated from the survey.

All zones and areas were visually censused using a face mask and snorkel on five different 2-d sampling periods during the summer months (June, July, and August) of 1982. All surveys were conducted between 10:00 and 15:00 using as closely as possible the following census system. The first zone to be surveyed each sampling occasion was selected at random. The observer swam down the centre of area A in the first zone counting all northern pike within an estimated 3-m strip width, area B in the adjacent zone, area A in the third zone, and so on until all zones were covered. The same procedure was carried out the following day on the complementary areas within each zone. Based on the clarity of the water, the crypticity and approachability of northern pike, and the screening effect of the habitat, a 3-m strip width was considered to be optimal.

Accuracy of the estimation of strip width (distance) and depths was assessed by comparing 20 estimated depths and 20 estimated distances within the littoral zone with the actual depths and distances as measured directly by hand to within 1 cm. There was no significant differences between the estimated distance and depth measurements and the actual distance and depth measurements. Thus, the estimation procedures used are considered appropriate for application to the strip census method. However, if ropes were placed down at some predetermined depth and a fixed distance apart, estimation of depth and distance would not be necessary.

Stratified systematic sampling was used because it is efficient in terms of the time and personnel required and tends to reduce the variability between samples, thereby increasing the precision of the estimate (Struhsaker 1981). It was assumed that movement of pike between and within transects would be minimal during each sampling period. A systematic sampling regime allows one to sample without replacement during each 2-d interval and avoids the logistical difficulties of random sampling (Caughley 1977).

Population estimates were calculated for sampling period 1; sampling periods 1 and 2 pooled; 1, 2, and 3 pooled; 1, 2, 3, and 4 pooled; and 1, 2, 3, 4, and 5 pooled, following formulae presented by Caughley (1977):

$$\hat{y} = RZ$$

where $\hat{y}$ = estimated total population, $R = \Sigma y_i / \Sigma z_i$, y_i = number of individuals on transect i, z_i = area of transect i, and Z = total area under survey.

The estimated variance of the population estimate was calculated using a formula presented by Caughley (1977):

$$V(\hat{y}) = (N^2/n)s^2$$

where $s^2 = \Sigma(y_i - \bar{y})^2/(n-1)$, N = total number of sampling units in the total area, and n = number of transects surveyed. This method of calculating the estimated $V(\hat{y})$ can be used for variable plot size sampled with or without replacement (Seber 1973; Caughley 1977). Caughley (1977) presented a more precise formula for the computation of the population variance for sample areas of differing size when the number of transects within a stratum is greater than 30.

Estimates were computed for each stratum separately. Caughley (1977) stated that the simplest means of combining estimates from more than one stratum is to sum the estimates for the strata and then sum the variances of the stratal estimates to give a variance for the combined estimate. As sample sizes within strata were relatively small (6–30), this conservative method was used to compute a combined estimate for areas A and B. Alternative analyses for producing a "weighted" variance were described by Cochran (1977). The standard error of each estimate was derived as the square root of the variance and used to produce approximate 95% confidence limits (Seber 1973; Caughley 1977).

Depth distribution — A major assumption in the stratified sampling design used is that the depth distribution of the northern pike remains constant throughout the period of investigation. An additional transect experiment was run concomitant with the population census to examine the depth distribution of northern pike and was used to validate this assumption.

Zone 1 was selected as the study site (Fig. 1). Transect lines of yellow polypropylene rope (marked off at 1-m intervals) were laid out parallel to the shoreline at four different depths from 0.6 to 4.8 m in representative habitat areas. One transect (depth = 0.6 m) was 200 m in length, the remaining three transects were 50 m in length (Fig. 1). In terms of vegetation characteristics, habitat was homogeneous along the length of each transect. A strip width of 3 m was used.

To examine month to month variation in the depth distribution of the adult pike (age 1 yr or older), each transect was surveyed using snorkel and SCUBA 12–15 times during the daylight hours in the last 2 wk of June, July, and August (approximately four times per month). Transect 1 was also surveyed at various times in the first 2 wk of July and August as part of the "test for independence" (outlined in a subsequent section). In addition, approximately 100 transects were surveyed across open-water portions of the lake at various depths throughout the entirety of the sampling period. No pike were observed in these offshore waters.

Depth distribution data were analyzed using a Kruskal–Wallis test for differences between transects in a given month and the Kruskal–Wallis multiple comparisons test for a posteriori comparisons between pairs of transects (Sokal and Rohlf 1981; Conover 1980).

Reaction of pike to observer — A major concern in visual surveys is duplicate counts due to observer error and missed counts due to the hiding or dispersal of alerted fish in front of divers. To examine these sources of error in our census, the gross behavioral reaction of observed pike to the presence of the diver was recorded for pike sighted on transect 1, the transect characterized by the highest abundance of pike. Reaction was categorized as follows: no reaction, a slow retreat, or a dart. Chi-square analyses indicated that reaction to a diver was independent of day in June, July, and August. These homogeneous samples were thus pooled, and data were then analyzed using a chi-square test for independence of pike reaction with respect to month.

Test of independence — An assumption that underlies repeated surveys of transects is independence of samples: the

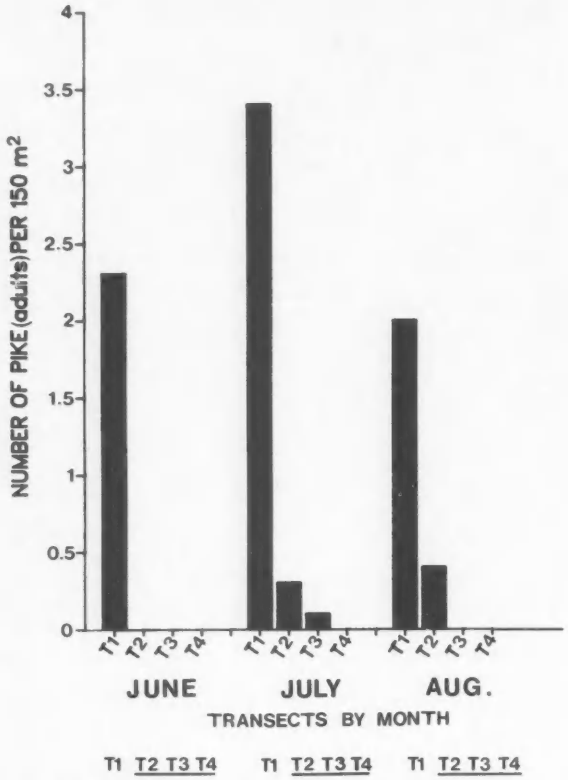

FIG. 2. Number of adult pike (age 1 yr or older) observed on transects in Roi Lake during the summer months. Data were analyzed using a Kruskal–Wallis test for differences between transects in a given month and the Kruskal–Wallis multiple comparisons test for a posteriori comparisons between pairs of transects. Transect symbols underlined are not significantly different at $P < 0.05$.

same fish should not be recorded on each census (Struhsaker 1981).

There was no evidence to indicate large-scale movements of pike throughout Roi Lake. However, qualitative observations in the preliminary survey suggested mixing of pike within a given area of the lake on a day to day basis. To assess the question of independence in a quantitative manner, a tagging study was carried out during the period of investigation. Forty northern pike (age 1 yr or older) were tagged with sphaghetti dart tags (Floy Manufacturers, Seattle, WA) with a different tag color used in each zone. Fifty percent of these fish were captured and released in the zone 1 study area. Each time transect 1 was surveyed, the number of tagged fish, the color of the tag, and the estimated size of each fish were recorded (size estimates were accurate to within ±5 cm). A tagged fish sighted with a total length within ±5 cm of a tagged fish with the same tag color sighted on the previous survey was considered to be a "repeat" observation. The ratio of repeat observations to the total number of tagged fish sighted was used as an estimate of repeatability.

Results and Discussion

Northern pike selectively use shallow habitat in Roi Lake during the daylight hours of the summer months (Fig. 2). There were significantly more pike on transect 1 than on transects 2, 3, and 4 during June, July, and August (Kruskal–Wallis tests, $P < 0.05$). Consistent use of shallow-water habitat throughout the summer months justifies the application of a stratified sampling regime for the estimation of population size. The littoral distribution of pike in Roi Lake is in general agreement with a number of distribution-related studies on the species. Diana et al. (1977), in an investigation of habitat use by northern pike in a relatively large, shallow, temperate lake, found that pike occupied water greater than 4 m in depth 5% of the time and were associated with vegetation 95% of the time (summer months). Pike in a large mesotrophic lake of central Alberta, although relatively versatile in their habitat use, selected shallow areas (Chapman and Mackay 1984a). Similarly, direct observation in two small, deep, pothole lakes in the same region indicated preferential use of shallow vegetated areas by northern pike (Chapman and Mackay 1984b; L. J. Turner, unpubl. data).

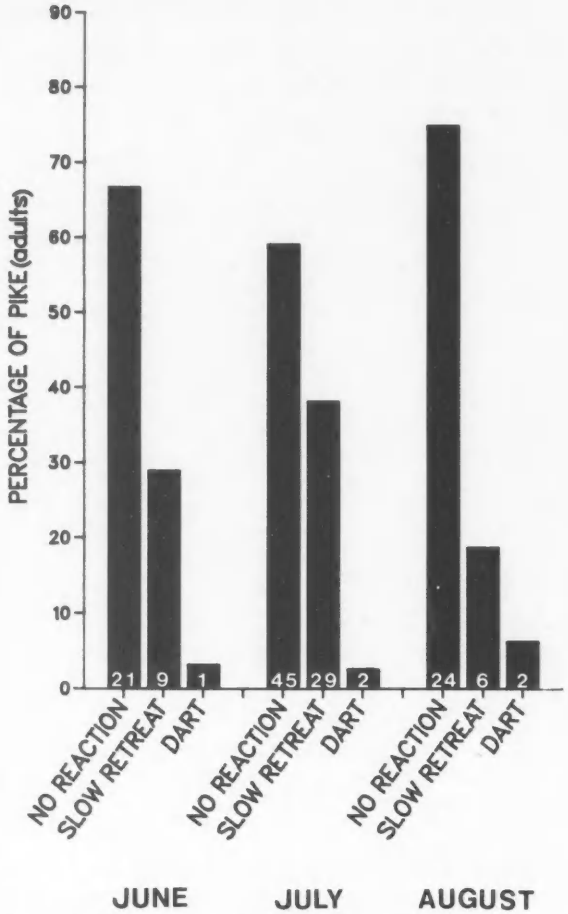

FIG. 3. Behavioral reaction of northern pike to the presence of an observer during June, July, and August in Roi Lake. The chi-square test was performed on the raw data (presented at the base of each bar); percentages have been used for graphic representation. H_0: reaction of northern pike to diver presence is independent of month, $\chi^2 = 4.5$, df = 4, $0.5 > P > 0.1$.

Reaction of pike to the presence of an observer was independent of month ($\chi^2 = 4.5$, df = 4, $0.5 > P > 0.1$, Fig. 3). No apparent behavioral reaction to diver presence was exhibited by

TABLE 1. Population estimates for northern pike in Roi Lake, summer months, 1982 (area A, <1 m; area B, 1–3 m; $\hat{y}$-estimated total population; $V(\hat{y})$-estimated population variance). Sample period: 1, June 24–25; 2, July 8–9; 3, July 15–16; 4, July 26–27; 5, August 18–19.

Sample	No. of transects per area	$\hat{y}$		$V(\hat{y})$		95% CI		
		A	B	A	B	A	B	A + B
1	6	155	3	1667	3	105	4	90
1 + 2	12	144	5	507	5	50	5	47
1 + 2 + 3	18	158	5	512	4	48	4	46
1 + 2 + 3 + 4	24	174	4	456	2	44	3	43
1 + 2 + 3 + 4 + 5	30	176	4	368	2	39	3	39

TABLE 2. Population densities of northern pike in various north-temperate waters. Where data were given as densities for different size or age classes, estimates are presented for pike age 1 yr or older. Population estimates given over a number of years are expressed as a range.

No. of pike/ha	Size of age classes included	Method	Lake or river	Year of estimate	Source
9	>25 cm	Rotenone	Loch Choin, Scotland	1955	Munro 1957
29	>14 in	Mark–recapture	Maple Lake, Minnesota	1957–58	Seaburg and Moyle 1964
46	>10 in		Grove Lake, Minnesota		
9	1+ or older	Gill net recaptures	Lake Windermere, England	1951	Kipling and Frost 1970
2–10	All sizes	Mark–recapture	Escanaba Lake, Wisconsin	1959–64	Kempinger and Carline 1978
10	>45 cm	Mark–recapture	Slapton Ley, England	1975, 1976–77	Bregazzi and Kennedy 1980
31–80	1+ or older	Mark–recapture	River Frome, England	1971–77	Mann 1980
23	1+ or older (>17 cm)	Strip census	Roi Lake, Alberta	1982	This study

64.7% of the observed pike, 31.7% retreated slowly into nearby cover, and 3.6% darted out of sight. Pike that exhibited a slow retreat were not considered to have been a source of error with respect to counts, as it was possible to monitor the position at which the fish finally settled. Pike that exhibited the "dart reaction" may have been a possible source of error; however, a 4% error is not a significant problem compared with the variability of the final estimates. To maintain a low level of disturbance it is necessary to census all transects at an extremely slow swimming speed (200 m/20–30 min). In addition, the observer must swim along the edge of emergent macrophytes, not through this type of vegetation. Qualitative observations indicated that any movement through the zone of emergent vegetation disturbed northern pike up to 5 m in front of the observer.

A total of 47 tagged fish were sighted in the surveys of transect 1 during the summer months. Six tagged fish were within ±5 cm of the total length of a marked fish (same tag color) sighted on the previous sampling occasion. This gives a maximum repeatability error estimate of

$$6/(47 - 4 \text{ (fish sighted on last census, no subsequent sample)}) \times 100 = 14\%.$$

Therefore, a maximum of 14% of the fish sighted on one census were likely observed in the subsequent survey. However, an average of 11 pike were sighted on transect 1 during each survey, approximately 6% of the total estimated population (refer to final estimate). Thus, if mixing between samples was complete, one would expect 6% repeatability. An additional 8% repeatability (as indicated in the test for independence) can be attributed to dependence between surveys, i.e. samples were 92% independent, which is an adequate degree of independence to justify the use of the transect census technique with respect to this assumption.

The final combined estimate for all five sample periods was 180 ± 39 pike (95% CI, Table 1). This estimate is well within the range of a number of estimates presented in published

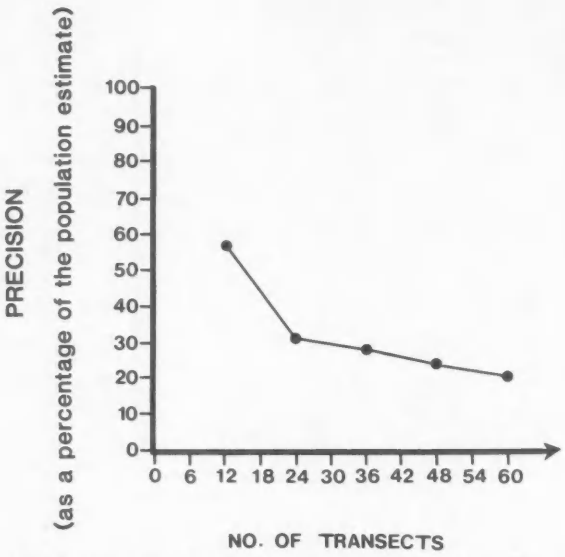

FIG. 4. Percentage precision of population estimates for northern pike (age 1 yr or older) in Roi Lake, based on an increasing number of transects. % precision = (95% CI/$\hat{y}$) × 100.

accounts (Table 2). A similar review of pike densities in north-temperate waters is presented in Bregazzi and Kennedy (1980). A problem with comparing densities of fish per surface area of the water body relates to the pattern of habitat use or depth distribution exhibited by pike populations. Pike often inhabit the shallow littoral zone, and Grimm (1981) presented evidence to indicate that the standing stock of northern pike which a water body can support is directly related to the surface area of aquatic vegetation rather than the surface area of the water body. As such, densities per unit surface area of the water body may not reflect densities per surface area of available habitat. The density estimate for pike in less than 3 m of water (96/ha) may more accurately represent the density of pike in Roi Lake.

The precision of a density estimate is usually expressed as the 95% confidence limits divided by the mean number of animals or groups per transect (Struhsaker 1981). However, as we have concentrated on population size rather than density, the precision of each estimate was calculated as the 95% confidence limits expressed as a percentage of the estimated population size. The precision curve (Fig. 4) allows one to estimate the required sample size that maximizes precision and minimizes effort. The curve tends to level off at approximately $n = 24$, suggesting that a sample in excess of 24 did not significantly improve the estimate.

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PERSPECTIVES

Reciprocal Oscillations in Densities of Larval Fish and Potential Predators: A Reflection of Present or Past Predation?[1]

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Frank, K. T., and W. C. Leggett. 1985. Reciprocal oscillations in densities of larval fish and potential predators: a reflection of present or past predation? Can. J. Fish. Aquat. Sci. 42: 1841–1849.

Reciprocity in time series data on the abundance of potentially interacting species has been one basis of empirical support for existing predator–prey theory. Evidence of this type has frequently been used to support the belief that predation by macroinvertebrates is one of the major causes of mortality among the early life stages of marine fishes. We question the validity of this generalization. We observed statistically significant inverse correlations between the abundance of macroinvertebrate predators and coastal ichthyoplankton in coastal Newfoundland both at the same site between years and at different sites in the same year. This correlation was shown to result not from a causal predator–prey interaction, but from occupation by the larvae and the macroinvertebrates of discrete water masses whose presence in the coastal area oscillates temporally in response to changes in wind conditions. Reevaluation of previously published reciprocal oscillations in the abundance of larval fish and potential predators, which had been cited as evidence of predatory regulation of larval numbers, showed that in all cases available physical data suggest that these correlations too may have been spurious. We suggest that historical temporal variations in predator abundance may have served as a template for the evolution of adaptive strategies on the part of larval fishes which effectively isolate them from potential predators in either the temporal or spatial dimension. Our analyses suggest that such adaptations involve active behavioral responses to reliable physical and/or biological signals indicative of the existence of ecological "safe sites."

La réciprocité des données sur les séries chronologiques décrivant l'abondance d'espèces possédant un potentiel d'interaction constitue une base d'appui empirique de la présente théorie prédateur–proie. On a souvent utilisé de telles preuves pour étayer l'opinion que la prédation de la part des macroinvertébrés est une des principales causes de la mortalité aux premiers stades vitaux des poissons marins. Les auteurs mettent cette généralisation en doute. Ils ont observé des corrélations inverses statistiquement significatives entre le nombre de macroinvertébrés prédateurs et l'ichtyoplancton peuplant les eaux côtières de Terre-Neuve, au même endroit d'une année à l'autre et à différents endroits pendant la même année. Les auteurs montrent que cette corrélation est le résultat de la présence de larves et de macroinvertébrés dans des masses aqueuses discrètes, présence qui oscille temporellement dans la zone côtière en fonction des variations du vent, et non d'une interaction causale prédateur–proie. Une réévaluation des oscillations réciproques de l'abondance des poissons larvaires et des prédateurs potentiels déjà publiées et qui avaient été citées comme preuves d'une régularisation du nombre de larves par les prédateurs a révélé que dans tous les cas, les données physiques disponibles portent à croire que ces corrélations sont peut-être également fausses. Selon les auteurs, les variations temporelles historiques de l'abondance de prédateurs peuvent avoir servi de base pour l'évolution de stratégies d'adaptation chez les poissons larvaires qui les isolent efficacement de prédateurs potentiels en milieu spatial ou temporel. Les analyses réalisées par les auteurs portant à croire que de telles adaptations font intervenir des réactions comportementales actives à des signaux biologiques ou physiques fiables qui indiquent l'existence de «milieux écologiques sûrs».

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The classical Lotka–Volterra predator–prey model produces reciprocal oscillations in predator and prey abundance familiar to all ecologists. Regular asynchronous fluctuations in the abundance of predator and prey among field populations are generally accepted as support of this theory. It has been argued that the widespread applicability of this theory has obstructed the development and testing of alternative hypotheses (Wangersky 1978; Larkin 1979). This is especially true for pelagic habitats where the effects of refugia and variations in food supply on predator–prey dynamics are considered to be minimal (Zaret 1980).

It is widely believed, for example, that predation by macroinvertebrates is a major cause of mortality among the early life stages of marine fishes (May 1974; Hunter 1976; Laurence 1981). Laboratory studies (Fraser 1969; Thielacker and Lasker 1974; von Westernhagen and Rosenthal 1976; Kuhlmann 1977; von Westernhagen et al. 1979; Arai and Hay 1982; Bailey 1984) have consistently shown that members of certain macroinvertebrate taxa (e.g. Chaetognatha, Ctenophora, Medusae, Crustacea) can consume large quantities of eggs and larvae when predator and prey are together in confined conditions. Field data are less convincing. Many difficulties beset the design and execution of field studies intended to assess the real quantitative impact of this myriad of invertebrate predators on larval fish. Consequently, the evidence presented to date in support of the predation hypothesis has been largely circumstantial: correlative studies showing inverse relationships between various macrozooplankters and ichthyoplankton (Pearcy 1962; Möller 1979, 1980a, 1984; Alvariño 1980), occasional extremely high densities of invertebrate taxa (Bigelow 1926; Meek 1928; Russell 1952), and the observation that the heaviest mortalities occur during the period when larvae are living on their yolk (Farris 1960). We remain skeptical. Reliable field estimates of larval fish mortality rates caused by predation are virtually nonexistent and the reported inverse correlations between numbers of predators and prey do not imply causality.

In this paper we draw on published and newly gathered data on the dynamics of macroinvertebrates and larval fishes to examine the validity of the assumption that negative correlations between densities of macroinvertebrates and larval fishes constitute reliable evidence of predation.

Site Specific Studies — Bryant's Cove, Newfoundland

During 1979 and 1980 we examined the short-term dynamics of the abundance of the dominant macrozooplankton at our Bryant's Cove, Newfoundland, study site (47°40.36′N; 53° 22.24′W) and of the eggs and larvae of 20 species of marine fishes of which the larvae of capelin (*Mallotus villosus*), winter flounder (*Pseudopleuronectes americanus*), radiated shanny (*Ulvaria subbifurcata*), and seasnails (*Liparis* spp.) constituted >95% by number. Capelin larvae hatch from adhesive eggs deposited intertidally at the heads of coastal embayments, while the other dominant larval species hatch from adhesive eggs positioned in the immediate subtidal region at depths <6 m. The macrozooplankton considered included three taxa: chaetognaths (primarily *Sagitta elegans*), jellyfish (most abundant were *Sarsia tubulosa*, *S. princeps*, *Catablema vesicarium*, *Aglantha digitale*, *Leukaritiara nobilis*, *Euphysa flammea*, *Aurelia* sp., and *Cyanea* sp.), and ctenophores (primarily *Pleurobrachia* sp.). These three taxa accounted for 92 and 74% of the total macrozooplankton fauna collected in 1979 and 1980, respectively. They are known, from experimental studies and direct observation of gut contents, to be predators of larval fishes (Table 1). Other taxa occurring in our collections, but not considered here, included decapod larvae, amphipods, and barnacle larvae.

All samples were collected with a 0.5-m-diameter plankton net fitted with a No. 10 (150-μm) mesh net and a General Oceanic flowmeter. Collections were made by towing the net horizontally (towing speeds averaged 1.2 m/s) in the 1- to 2-m depth strata along six transects running parallel to the beach at Bryant's Cove. The first transect was located 15 m from the beach in relatively shallow water (3-m depth). The remaining five were separated by 140 m, and terminated 715 m from the beach where the water depth in the cove is maximum (22 m). Each transect spanned the width of the cove (70–200 m). A second set of four transects, oriented perpendicular to the beach and extending from a point 15 m from the beach to a second point 715 m from the beach, was sampled coincident with the transects running parallel to the beach during 1979. Samples were collected between 18:00 and 20:00 and were preserved in 4% buffered formalin. All fish larvae and macrozooplankton were sorted from the sample and counted.

Comparison of the density of ichthyoplankton between pairs of adjacent transects oriented parallel to the beach revealed extremely high spatial similarity (range of r: 0.85–0.95, $p < 0.001$) during the June–August study period in both 1979 and 1980. This spatial similarity in density estimates between transects was also characteristic of the macrozooplankton component (range of r: 0.82–0.95, $p < 0.001$). Within-day variation among the six parallel transects was also low. Coefficient of variation (CV) values calculated on the untransformed densities averaged 59 and 61% for the ichthyoplankton and macrozooplankton, respectively.

Weighted average densities of ichthyoplankton and macroinvertebrates collected on the transects oriented parallel and perpendicular to the beach were virtually identical (ichthyoplankton: slope = 0.99, $Y_{int} = 0.37$, $r = 0.95$, $p < 0.001$; macroinvertebrates: slope = 0.92, $Y_{int} = 0.37$, $r = 0.97$, $p < 0.001$).

Collectively, these data indicate that the distribution of ichthyoplankton and macrozooplankton in Bryant's Cove is homogeneous at space scales ranging from approximately 50 to 1000 m. This conclusion is supported by previous work (Frank and Leggett 1981, fig. 4) which showed that changes in densities of larval capelin at intertidal stations spaced at 50-m intervals across the beach at Bryant's Cove were synchronous. We therefore used weighted average densities of larvae and invertebrates, calculated from samples collected on the parallel transects during 1979 and 1980, as descriptors of the abundance of macroinvertebrates and ichthyoplankton in the cove at large.

During both 1979 and 1980, peaks in the abundance of ichthyoplankton were followed by peaks in the combined abundance of the three predator taxa. This pattern, evident in both years of the study, occurred at different times during the summer within each year (Fig. 1) and was reflected in significant inverse correlations between the abundance of predator and prey during both years (Table 2). Reciprocal predator and prey abundance was the dominant pattern in the plankton community during the month of July for *both* 1979 and 1980. July is also the period of maximum abundance for larvae of capelin, winter flounder, radiated shanny, and seasnails (Frank and Leggett 1983a; Leggett et al. 1984). The slope of the relationship between predator and prey was consistently negative during the months of June and August, but the number of significant

TABLE 1. Studies confirming the occurrence of fish eggs and larvae in the diet of jellyfish, chaetognaths, and ctenophores and their estimated feeding rates on fish larvae under experimental conditions.

Field studies		Laboratory studies	
Predator	Source	Feeding rate	Source
Jellyfish (e.g. *Cyanea*, *Aurelia*, *Sarsia*, *Leuckartiara*, *Staurophora*, *Aequorea*, *Phialidium*)	Gudger 1934 Russell 1953 Stevenson 1962 Fraser 1969 Phillips et al. 1969 Larson 1976 Möller 1984	6–300 larvae/d	Fraser 1969 Arai and Hay 1982 Bailey and Batty 1983 Bailey 1984
Chaetognaths (e.g. *Sagitta elegans*, *S. setosa*)	Lebour 1922, 1923 Bigelow 1926 Thomson 1947 David 1955 Furnestin 1957 Stevenson 1962	0.3 larvae/d	Kuhlmann 1977
Ctenophores (e.g. *Pleurobrachia* spp.)	Lebour 1922, 1923 Bigelow 1926 Russell 1935 Fraser 1962, 1970 Stevenson 1962	8 larvae/d	Frank and Leggett 1982[a]

[a]An in situ field experiment.

correlations in these months relative to July was reduced, at least in part due to low sample sizes. Ichthyoplankton abundance was low (typically 1 to 2 orders of magnitude below peak numbers) when predator densities exceeded $10/m^3$. By previously accepted criteria (Table 3) this reduction in ichthyoplankton density would serve as indirect support for the predation hypothesis, and for the argument that predator abundance is an important regulator of larval survival.

We have previously shown, however, that temporal variations in the abundance of both the ichthyoplankton and macrozooplankton complexes at Bryant's Cove are correlated with the periodic occurrence of two distinct water masses in the nearshore area. Macrozooplankton densities are greatest when cold subthermocline water upwells in and dominates the nearshore zone during offshore wind conditions (Frank and Leggett 1982). The similarity in water temperatures in Bryant's Cove and in the offshore, subthermocline waters of Conception Bay (waters below 20 m) during offshore winds confirms the source of the cold water mass that dominates the nearshore during these conditions (Fig. 2). The vertical distributions of the dominant macroinvertebrate taxa in our study area (jellyfish, chaetognaths, and ctenophores) are known to be restricted to thermocline and subthermocline waters (Table 4). This explains our finding that their numbers are limited in the nearshore during onshore winds when warm surface waters dominate the area, and that a rapid and dramatic increase in their numbers in the nearshore occurs during coastal upwelling that accompanies offshore winds. Temporal changes in the abundance of these macroinvertebrates in Bryant's Cove thus result from a combination of advection and the elimination of thermal barriers to vertical migration.

In contrast, the ichthyoplankton densities are maximal when warm surface water masses (<15 m; Fig. 2) are driven landward and dominate the nearshore during periods of onshore winds. Temporal variations in the abundance of the dominant elements of the midsummer ichthyoplankton (capelin, winter flounder, radiated shanny, and seasnails) result from active behavioral responses to reliable environmental signals (presumably temperature) that accompany wind-driven water mass exchange (Frank and Leggett 1983b). These behaviors cause the larvae to terminate their demersal existence and to initiate their drift in the warm surface water mass which is both rich in food and relatively depauperate of predators (Frank and Leggett 1982).

This interpretation of the independence of temporal changes in larval fish and macroinvertebrate numbers is supported by our finding that at our Bryant's Cove study site, temporal shifts in the abundance of both the macroinvertebrate and ichthyoplankton complexes were more highly correlated with wind effects indicative of nearshore water mass characteristics than with one another (Table 5). Moreover, the negative correlations between macroinvertebrate and ichthyoplankton densities could not have resulted from seasonal trends in the abundance of jellyfish, ctenophores, and chaetognaths because no such trends existed ($r^2 < 0.04$; Frank and Leggett 1982). This indicates that the reciprocal variation in macroinvertebrate – larval fish densities reported here was more the result of spatial/temporal separation resulting from association of fish larvae and macroinvertebrate predators with different water masses than of interaction between the two species complexes.

We conclude that the inverse correlation between the abundances of macroinvertebrates and larval fishes illustrated in Fig. 1 and Table 2 results not from direct biological interaction, but from an active response by the ichthyoplankton to wind-driven changes in the character of the coastal water mass which effectively isolates them from potential macroinvertebrate predators. The key to the timing of this response is the occurrence of temperature or other signals associated with wind-driven water mass exchange.

Coastwide Studies — Eastern Newfoundland

In July 1982, in order to assess the scale of these associations between water mass exchange and macroinvertebrate/ichthyoplankton dynamics, we sampled the plankton simultaneously at

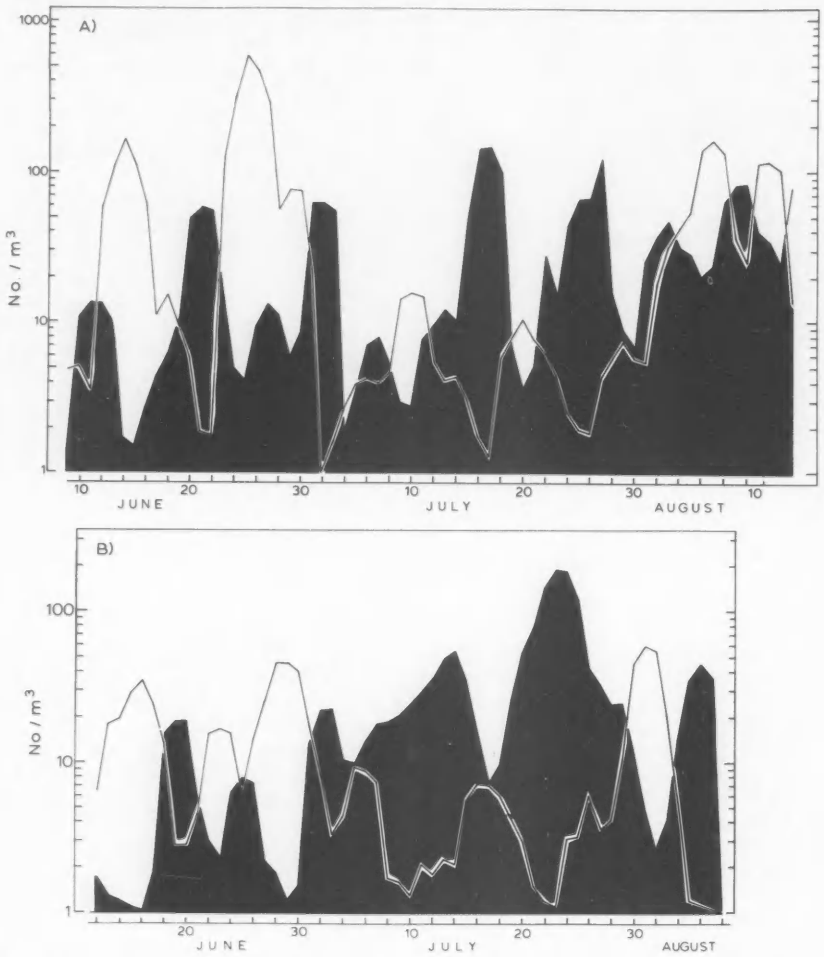

FIG. 1. Day-to-day changes in the abundance of ichthyoplankton (shaded curve) and invertebrate predators (unshaded curve) at the Bryant's Cove, Newfoundland, study site during June–August for (A) 1979 and (B) 1980. Missing values were calculated by using the equations contained in Table 5, and the resulting time series were smoothed using a 3-d moving average to illustrate the apparent reciprocal trends in abundance of the two groups. Is this really evidence for predator regulation of ichthyoplankton numbers or is predator abundance acting as an environmental template for the evolution of adaptive avoidance behavior?

Bryant's Cove and at Bellevue Beach, Trinity Bay, and at Eastport, Bonavista Bay; these latter are located 75 and 250 km, respectively, north from Bryant's Cove. At both sites, macrozooplankton and larval fishes (dominated by capelin larvae) were sampled at three equally spaced intertidal stations with a rectangular mouth (20 × 29 cm) No. 10 mesh plankton net attached to a clear Plexiglas sled and frame (for a more complete description of this gear and its use see Frank and Leggett 1981). At Bryant's Cove and Eastport, samples were collected daily; at Bellevue they were collected twice weekly.

Significant negative correlations, similar to those previously described for Bryant's Cove (Table 2), were observed between ichthyoplankton and macrozooplankton densities at Eastport ($r = -0.52$, $n = 22$, $p < 0.05$) and Bryant's Cove ($r = -0.60$, $n = 22$, $p < 0.01$). The slope of the relationship between the two components was negative but nonsignificant at Bellevue Beach ($r = -0.40$, $n = 12$, $p > 0.05$), in part due to a low sample size. At all three locations the abundance of macrozooplankton (jellyfish, ctenophores, and chaetognaths) was strongly and positively correlated with the occurrence of offshore winds (Bellevue Beach: $r = 0.76$, $n = 12$, $p < 0.01$; Eastport: $r = 0.72$, $n = 22$, $p < 0.001$; Bryant's Cove: $r = 0.75$, $n = 22$, $p < 0.001$). Furthermore, daily fluctuations in macrozooplankton densities at Eastport and Bryant's Cove were synchronous ($r = 0.54$, $n = 17$, $p < 0.05$; Fig. 3B). During 1982, fluctuations in larval capelin abundance at Eastport were synchronous with those recorded at Bryant's Cove ($r = 0.79$, $n = 17$, $p < 0.01$; Fig. 3A), and at both locations between 53 and 72% of the daily variation in larval density was explained by noncyclic wind events, maximum larval densities occurring consistently during periods on onshore winds. In spite of the small sample sizes at Bellevue Beach, larval density and wind events were signifi-

TABLE 2. Correlations between predator (macroinvertebrate density; ln no./m^3) and prey (ichthyoplankton density; ln no./m^3) by month for 1979 and 1980 and for both years combined (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

	June			July			August		
Predator–prey	1979	1980	Σ	1979	1980	Σ	1979	1980	Σ
Y_{int}	2.47	2.69	2.62	3.61	3.60	3.51	4.02	3.01	3.29
Slope:	−0.258	−0.756	−0.414	−0.932	−0.565	−0.658	−0.199	−0.859	−0.153
r	−0.49	−0.81	−0.65*	−0.55*	−0.63**	−0.59***	−0.25	−0.98*	−0.27
n	9	4	13	13	23	36	4	4	8

TABLE 3. Evidence from the field showing that the distribution of prey and predator are correlated. This was the basis of support for the hypothesis that predation by macroinvertebrates is an important source of larval mortality.

Source	Prey	Predator	Type of analysis
Strasburg 1960	Tuna	Macroplankton	Eye fit (−) curve
Pearcy 1962	Winter flounder	*Sarsia*	Lag correlation (+)
Ali Kahn and Hempel 1974	Assorted larvae	Chaetognaths, salps, ctenophores	Eye fit (−) curve
Alvariño 1980	Anchovy	Chaetognaths, medusae, siphonophores, ctenophores, chondrophores	(−) relation for 3 yr
Möller 1979	*Gobius* spp.	*Aurelia*	(−) correlation
Möller 1980a, 1984	Herring	*Aurelia*	(−) correlation
Arai and Hay 1982	Herring	*Sarsia, Aequorea*	Time series: prey, predator
van der Veer and Zijlstra 1982	Plaice, flounder	*Pleurobrachia, Sarsia*	Time series: prey, predator

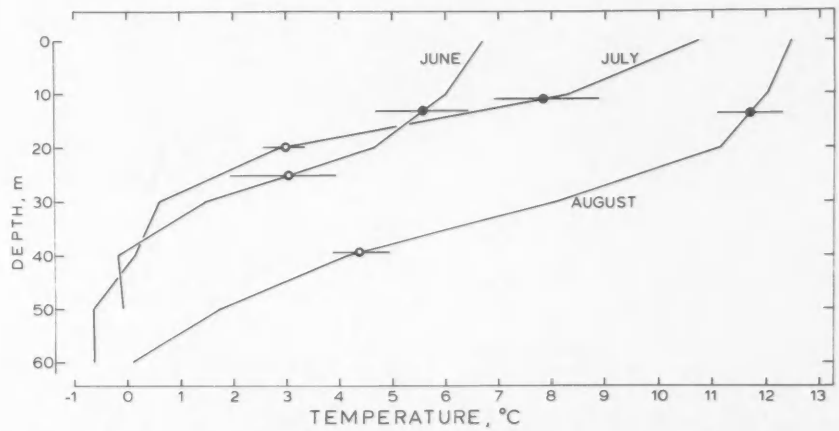

FIG. 2. Composite temperature–depth profile by month for the offshore waters of Conception Bay, Newfoundland. Values for temperature are means based on Nansen bottle and bathythermograph data supplied by the Marine Environmental Data Service (Ottawa) in the area bounded by 47°28′N–47°50′N, 52°50′W–53°10′W. Superimposed on each monthly temperature–depth profile are the mean monthly depth averaged temperatures measured in Bryant's Cove during onshore (●) and offshore (○) wind conditions for 1979 and 1980. The narrow range of the means of temperature in the cove at 1, 10, and 20 m, denoted by the horizontal lines, suggests that the nearshore waters are well mixed under both wind conditions.

cantly correlated ($r = 0.56$, $n = 12$, $p < 0.05$), and fluctuations in ichthyoplankton abundance were near synchronous with those recorded at Bryant's Cove ($r = 0.57$, $n = 9$, $p < 0.25$).

Simultaneous continuous records of nearshore water temperature at the three capelin spawning sites, made in 1982, showed that water temperature changes at all sites were virtually synchronous (Fig. 4) and highly correlated (Eastport–Bellevue: $r = 0.56$, $p < 0.01$; Eastport – Bryant's Cove: $r = 0.55$, $p < 0.01$; Bellevue – Bryant's Cove: $r = 0.41$, $p < 0.05$; $n = 31$). Each of the major peaks in macrozooplankton density (>40/m^3) that occurred synchronously between Eastport and Bryant's Cove during 1982 (Fig. 3) coincided precisely with periods of falling water temperatures (Fig. 4). In addition, the three concurrent peaks in ichthyoplankton density evident between

TABLE 4. Macroinvertebrates that associate with the discontinuity layer (thermocline and/or halocline) or tend to be confined to depth strata below such zones. Evidence presented is based on diel, discrete depth sampling, experimental water column studies, or observations of mass occurrences in coastal waters during upwelling events.

	Source
Jellyfish	
Aglantha digitale	Hansen 1951; Lie et al. 1983; Southward and Barrett 1983
Sarsia tubulosa	Hansen 1951; McCormick 1969; Arai 1973, 1976
Aurelia aurita	Kubawara et al. 1969; Möller 1979
Sarsia spp.	McCormick 1969
Phialidium gregarium	McCormick 1969; Mills 1984
Rathkea octopunctata	McCormick 1969
Some S. Atlantic medusae	Moreira 1973
Many British Isles medusae	Russell 1970
Chaetognaths	
Sagitta elegans	Meek 1928; Hansen 1951; Harder 1968; Lie et al. 1983; Southward and Barrett 1983
Ctenophores	
Pleurobrachia spp.	Arai 1973, 1976; Möller 1979; Mills 1984

TABLE 5. Correlations of predator density (ln no./m^3) and prey density (ln no./m^3) with onshore wind frequency, expressed as $\sin^{-1}$ (h of onshore wind/d/24)$^{0.5}$ (*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).

	June			July			August		
	1979	1980	Σ	1979	1980	Σ	1979	1980	Σ
Predator–wind									
Y_{int}	5.10	3.54	4.68	2.10	3.03	2.38	5.71	2.49	4.33
Slope	−0.116	−0.081	−0.107	−0.045	−0.083	−0.061	−0.112	−0.056	−0.082
r	−0.79**	−0.84	−0.78**	−0.75**	−0.69***	−0.66***	−0.97*	−0.95*	−0.87**
n	9	4	13	13	23	36	4	4	8
Prey–wind									
Y_{int}	0.52	−0.42	0.27	1.02	1.51	1.19	2.75	0.52	2.10
Slope	0.062	0.082	0.067	0.090	0.063	0.075	0.051	0.054	0.031
r	0.81**	0.90	0.82***	0.87***	0.66***	0.78***	0.57	0.95*	0.57
n	9	4	13	13	23	36	4	4	8

the two locations (Fig. 3) were strongly linked to periods of rising water temperature (Fig. 4). From these findings we conclude that wind-driven water mass exchange, and the corresponding changes in macroinvertebrate and ichthyoplankton densities recorded at Bryant's Cove, Bellevue, and Eastport, occur characteristically and synchronously *over large expanses of the east coast of Newfoundland*.

Previously Reported Predator–Prey Interactions — A Reassessment

The results reported have led us to examine more closely the predator–prey interactions postulated in the studies detailed in Table 3. If our study had failed to examine the dynamics of the physical environment and had considered only fish larvae and macroinvertebrates, the highly significant negative correlation between larval fish and macroinvertebrate numbers might have been incorrectly interpreted as evidence of predatory regulation of ichthyoplankton density. Our reanalysis of these previously published studies led us to conclude that failure to consider the importance and influence of other environmental variables has contributed to incorrect conclusions being drawn about the causes of reciprocal changes in potential predator–prey numbers reported. Work overlooked by the investigators cited in Table 3, or published subsequent to their analyses, show that in most of the upwelling zones or coastal embayments studied the distribution and abundance of predator and/or prey are strongly influenced by meteorological and/or hydrographic factors.

Strasburg (1960) showed that the combined abundance of larval skipjack (*Katsuwonus pelamis*) and yellowfin tuna (*Thunnus albacares*) in the central Pacific was inversely correlated with invertebrate plankton volume at 30 stations within a 3 × 3° grid centered on the Hawaiian Islands. Subsequent work in this area (Shomura and Nakamura 1970) revealed significant positive correlations between zooplankton and physical factors such as salinity and the thickness of the isothermal layer. Miller (1979), working in the same area, found that wind-driven upwelling along leeward coasts in the Hawaiian Islands caused large numbers of tuna larvae, normally concentrated near the thermocline, to be advected to the surface. The result was higher densities of tuna larvae on leeward as opposed to windward coastal stations.

Ali Kahn and Hempel (1974) observed an inverse relationship between total zooplankton volume and fish larvae in the Gulf of Aden, Indian Ocean, prior to and during the northeast monsoon. They concluded that larval mortality due to predation was very high at this time. It is known, however, that the current regime and thermal structure of the waters in this area change significantly during the monsoon period. This directly affects nutrient levels and zooplankton production (Düing 1970; Currie

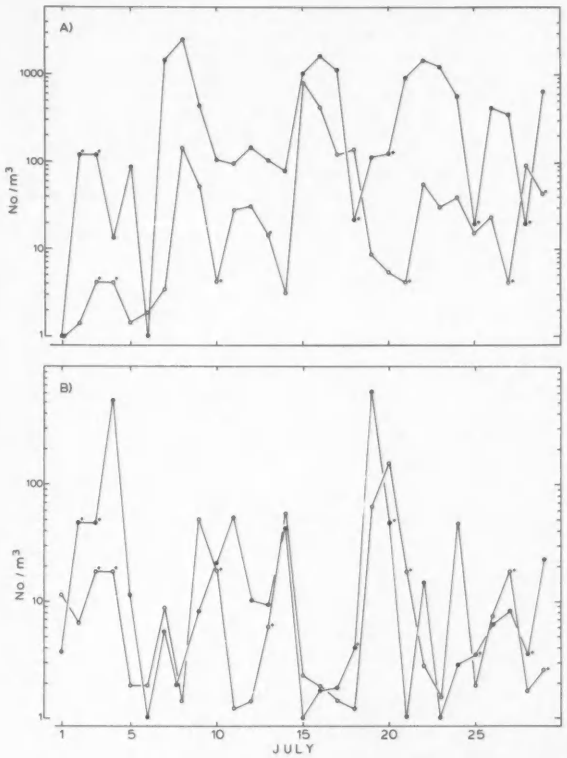

FIG. 3. Temporal changes in the abundance of (A) fish larvae and (B) macrozooplankton from Bryant's Cove (○) and Eastport (●) during July 1982. Equations used to predict larval density (Y_1) and macrozooplankton (Y_2) from onshore wind frequency (X) are as follows: Eastport, $\ln Y_1 = 4.83 + 0.064 \sin^{-1} (X)^{0.5}$, $\ln Y_2 = 3.84 - 0.090 \sin^{-1} (X)^{0.5}$; Bryant's Cove, $\ln Y_1 = 1.44 + 0.104 \sin^{-1} (X)^{0.5}$, $\ln Y_2 = 2.89 - 0.093 \sin^{-1} (X)^{0.5}$. A plus sign indicates values predicted from onshore wind frequency.

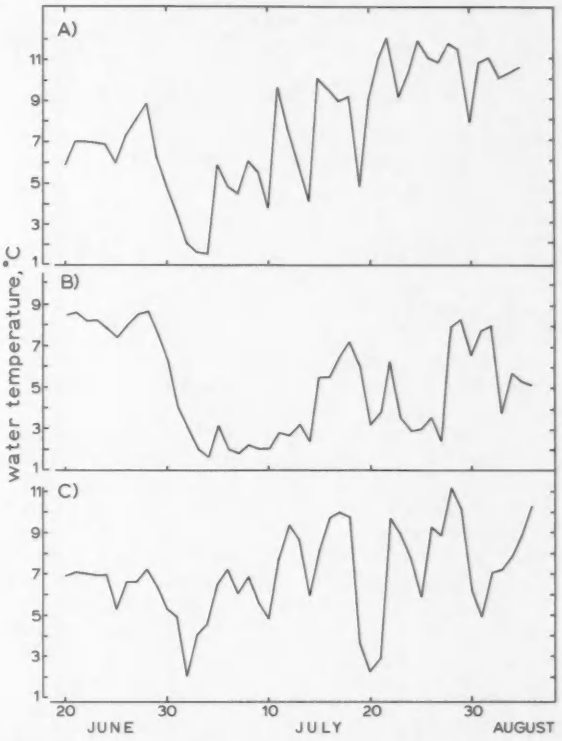

FIG. 4. Simultaneous record of nearshore water temperature adjacent to three capelin spawning sites in eastern Newfoundland (A, Eastport; B, Bellevue; C, Bryant's Cove), 20 June – 5 August 1982.

et al. 1973; Ali Kahn 1976). Because their data were not stratified into time periods (e.g. the periods characterized by the monsoon), the causal factors producing the inverse relationship between zooplankton and fish larvae remain obscure.

Alvariño (1980) analyzed 3 yr of CalCOFI data that showed an inverse relationship between the combined abundance of five major groups of large invertebrate predators and the abundance of anchovy (*Engraulis mordax*) larvae and concluded that planktonic predators significantly reduce local populations of larval anchovy. The California Current region is, however, characterized by localized upwelling events resulting in high interstation variability (Smith 1981). Hunter (1981) later suggested that anchovy spawn intensely in the California Current mosaic only in areas lacking large planktonic predators and rich in foods for the adults and larvae.

Möller (1979) reported a significant inverse correlation between the abundance of *Aurelia* and goby (*Gobius* spp.) larvae in Kiel Bight. In this instance it was shown that the abundance of *Pleurobrachia* and *Aurelia* in the inshore waters of the Kiel Bight depended on local winds (in particular, offshore winds induced upwelling that caused their mass occurrence near the coast, a situation identical to that which we observed in eastern Newfoundland). The correlation between the occurrence of offshore winds and the relative abundance of medusae and ctenophores in the Kiel Bight was positive and highly significant ($r = 0.82$, $p < 0.01$). There was, however, no corresponding adjustment for the effect of winds on local predator abundance in the development of Möller's predator–prey relationships. Möller (1980a, 1984), again working in the Kiel Bight area, showed that the biomasses of larval herring (*Clupea harengus*) and *Aurelia aurita* were inversely correlated. Möller (1984) concluded that the abrupt decline in the seasonal peak in larval herring abundance (sampled at weekly intervals for 2 mo each year) resulted from predation by the rapidly growing jellyfish population indigenous to the Bight and suggested that the predation effect superseded spawning stock size in determining the abundance of the larval herring population. However, results contained in a separate paper (Möller 1980b) dealing exclusively with the population dynamics of *Aurelia* suggested that outdrifting into the open Kiel Bight was responsible for some of the abrupt declines in *Aurelia* abundance witnessed. This is especially evident in Möller's fig. 1 (1984) where *Aurelia* biomass shows pronounced oscillations independent of larval herring abundance in each year of the 4-yr study. Once again, the possibility that wind regulation of predator abundance contributed to the observed correlation between predator and prey (Möller 1980a, 1984) was not considered.

Discussion and Conclusions

We conclude that the reciprocal oscillations in macroinvertebrate and ichthyoplankton densities detailed in Fig. 1 and

Table 2 are not consistent with the predator–prey interactions hypotheses advanced to explain them. Rather, the available evidence strongly suggests that they result from the occupation of distinct water masses by these two separate components of the plankton.

These findings lead us to conclude that the correlative approach to assessing the existence and significance of predator–prey interactions involving larval fish should be abandoned unless concurrent study is made of the physical components of the system of which both the proposed predator and prey are a part. The relationships between populations of both predator and prey and temporally or spatially ordered physical systems must be determined prior to any attempts to analyse direct predator–prey interactions.

Our analyses also lead us to conclude that increased sampling frequencies, which we employed and which have been regarded by several investigators (Marr 1956; Farris 1960; Gulland 1965; Smith 1978; Sharp 1981; Bakun et al. 1982) as essential to a better understanding of predator and prey dynamics, will not *in the absence of relevant physical data* yield meaningful advances in the understanding of biotic interactions between potential predators and prey.

Finally, we suggest that a new view of negative correlations between potential predators and their prey may be in order. It is entirely possible that many of the previously reported negative correlations reflect, as appears to be the case for capelin and other nearshore spawners in eastern Newfoundland, an adaptive response by the spawning adults or the larvae to historical patterns of predation. The contribution of such patterning to fitness will depend on the extent of coevolution of the predator in response to adaptations by the prey. The potential for such coevolution will, of course, be determined by the degree to which the predator exploits alternate prey to meet its energy requirements. Negative correlations based on adaptive responses would, under these criteria, be most evident between prey whose densities are temporally or spatially patchy and predators that are omnivorous feeders. The fact that significant concentrations of larval fish are known to be short lived and heterogeneous in time and space, and that those macroinvertebrates most frequently cited as potentially important predators of larval fish are, without exception, omnivorous (Tables 1, 3, 4), is consistent with this hypothesis.

Acknowledgements

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OBITUARY/NÉCROLOGIE

Henry Benedict Hachey
1901–1985

Dr. H. B. (Harry) Hachey, MBE, ED, M.Sc., LL.D., FRSC, one of the early Canadian pioneers in oceanography, died on June 24, 1985, at the age of eighty-four.

Harry Hachey was one of those rare individuals who combined within a lifetime several distinguished careers in science, military service and public administration. His advice and criticism thus drew on an exceptional breadth of knowledge and experience, always punctuated with humourous anecdotes to make a point clear. He provided the scope for personal initiative and only asked in return that the work be professionally conducted. Considerate and thoughtful of others, with his blend of humour and stories, he could criticize, scold and praise in such a way that all could regard him as a close friend and trusted colleague. His life was in harmony at all times, whether at the office, out hunting or with his family. He was warm and generous in response but quick to rise if affronted. He was free of complexities so that one was never in doubt about his views or beliefs. His life and work exemplified the leadership role that he accepted for almost half a century.

Born at West Bathurst, New Brunswick, in 1901, Dr. Hachey was a descendant of a distinguished Acadian family whose roots were first established in Lower Canada over 400 years ago. After growing up in the Bathurst area and attending St. Thomas University in Chatham, New Brunswick, he entered St. Francis Xavier University from which he graduated with a B.Sc. in 1922, before going on to obtain a M.Sc. degree from McGill University in 1925. There, he began his professional teaching career as a demonstrator and tutor, later to become a professor of physics at the University of New Brunswick, in Fredericton. His careers as scientist and as champion of national oceanography in Canada remained linked closely with the universities, being recognized with an LL.D. from both St. Thomas and St. Francis Xavier Universities. He was further elected a Fellow of the Royal Society of Canada in 1950, and included in the Queen's Honour List for the award of the Silver Jubilee Medal in 1977.

While at the Atlantic Biological Station as a summer investigator, in 1927, Harry met Dr. Henry Bigelow, who later became Director of the Woods Hole Oceanographic Institution, and was then preparing a national report on a worldwide program of oceanographic research for the United States. The meeting with Bigelow was sufficiently exciting for Harry that he abandoned his academic career and launched himself into a new life as an oceanographer. It was a wise decision in retrospect, for at the young age of 29 he was asked to lead the Hudson Bay Fisheries Expedition to determine whether or not a deepwater fishery of commercial importance existed in Hudson Bay. He joined the coal-burning trawler *Loubyrne* in Halifax in the summer of 1930, equipped with two reversing water bottles and thermometers and hand winches for plankton tows. The importance and expectations of opening up a new fisheries in this frontier area can only be guessed now, but as scientist-in-charge, he was obliged to report their findings daily, in code, to Ottawa.

Despite the disappointing fisheries results and inadequacies of the technology of the era, the research findings on water conditions, circulation, plankton and bottom fauna remained as classics and the only definitive source of oceanographic information on Hudson Bay until after the Second World War. The first update of his findings was undertaken, by himself and a colleague, in 1946 on the occasion of a naval expedition into Hudson Bay, by HMCS *Haida*, *Nootka* and *Magnificent*.

The advent of the depression years and a fire that destroyed the Atlantic Biological Station, St. Andrews, New Brunswick, spelled lean times for oceanographic research on the East Coast. Harry learned "to make do" in those hard times, going to sea in mere cockle shells by today's standards, but time was available for study, analyses and publication of major papers on the Labrador and Scotian Shelf water masses, Bay of Fundy and Gulf of St. Lawrence.

His intimate knowledge and expertise of East Coast waters

became invaluable later on in World War II, as military life was already familiar to him. Known to many as Colonel Hachey, he had maintained an active career in the army since joining first the Non-Permanent Active Militia in 1921 at the age of 20, readily answering the call to arms in 1940 by joining the North Shore New Brunswick Regiment, with the rank of Captain. He served overseas for 2 years on active duty, and on return to Canada was appointed General Staff Officer in chemical warfare research at Suffield, Alberta, and Lieutenant Colonel with the Army Operational Group. But with the submarine menace now at its worst off the Atlantic coast, Colonel Hachey was duly seconded from the army to the Royal Canadian Navy for special antisubmarine duties.

Again, headquartered at the Atlantic Biological Station, he had access to the extensive records of water conditions, largely compiled and organized by himself over the years for the Fisheries Research Board of Canada. With several naval officers, he set up the Atlantic Oceanographic Research Group (AORG) and his team served on the first Escort "Killer" Group (EG16) of the Royal Canadian Navy fitted with the most modern submarine detection and weapons systems of the day. Vital information was thereby provided to the Navy, based on the behaviour of the sound ranging equipment adapted for the highly stratified waters of the East Coast. It was both trying and demanding, with success being measured on the statistics of ship losses due to German action. At the close of hostilities, Harry came into the possession of U-boat atlases and, to his surprise and dismay, found many of his research publications, illustrated in full colour, reproduced in German and marked "Secret". However, by 1946, Harry was honoured by King George VI as a Member of the Order of the British Empire (MBE) in recognition of his special wartime services, and on discharge he was awarded the Efficiency Decoration (ED) in recognition of 20 years of meritorious service in the Canadian Army.

After the war, Harry returned to the Fisheries Research Board of Canada and headed up the Atlantic Oceanographic Group and in addition launched a new direction in Canada for oceanographic research as Chief Oceanographer for Canada. Initially, and with his wartime colleagues, he strove to unite and coordinate the work of various government organizations that had interests in using or contributing to the science of oceanography. His perseverance led to the formation of the Joint Committee on Oceanography, which he served as Secretary from 1946 to 1964, and later on the expanded Canadian Committee on Oceanography (CCO). Through his direct intervention, briefs and reports, he forged a government and university partnership that was instrumental in bringing a national focus and high international reputation to the marine sciences in Canada.

The evolution of the Canadian Committee on Oceanography required Dr. Hachey to steer a course through often long and stormy negotiating sessions, but his diplomacy, good humour and well-prepared and organized approach persevered to the lasting benefit of succeeding generations of Canadians. To those who were associated with him in the formative years of oceanography in Canada and to the many that have followed, it would be this accomplishment that would stand alone as a milestone in the history of Canadian oceanography, for it not only paved our way but also launched Canadian marine science into international and intergovernmental ocean science and affairs.

Over the years, Dr. Hachey was associated with many local committees and groups, civic and political. He served 13 terms as mayor of St. Andrews and 10 terms on the Charlotte County Council. He was President of the Charlotte County Liberal Association and contested the riding as a Federal Liberal candidate. He was active as Director and member on two hospital boards, Chairman of the Board of the Ross Memorial Museum and President of the Passamaquoddy Branch of the Royal Canadian Legion.

To his wife, Katherine Avis Cox, and his family Osmund, John, Mary-Isabel and Jane, he had much love as a father, counsellor, historian and poet. Many have shared some part of Harry's life with him and will remember those times with fondness. Many others of yet another generation are still to be touched by his foresight, perseverance and contribution to resolving the mysteries and sciences of the oceans of Canada, and to their future uses. Still others knew him as mayor of St. Andrews, civic leader and representative on the Charlotte County Council, where he set a standard of excellence and integrity to be envied by all.

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BOOK REVIEWS/COMPTES RENDUS

Fishes of the North-Eastern Atlantic and the Mediterranean/ Poissons de l'Atlantique du Nord-Est et de la Méditerranée. *Edited by P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E. Tortonese. Unesco Publications No. 56, Paris. 1984. Volume I, 510 p. fr250.*

This is the first of three volumes of *Fishes of the north-eastern Atlantic and Mediterranean* (FNAM), the long-awaited authoritative ichthyofauna of the marine fishes of Europe. It is the progeny of Clofnam (*Check-list of the fishes of the north-eastern Atlantic and of the Mediterranean*). The geographic area covered by FNAM extends from eastern Greenland to Europe, north and east to Spitzbergen and western Novava Zemlya, south to 30°N including the Azores, Madeira, most of Morocco, all of the Mediterranean, Black and Caspian seas.

FNAM is divided into a Foreword (by Théodore Monod), Editor's note, List of contributors (73 scientists), List of families (all three volumes), Key to families (all three volumes), and 64 family accounts with contained species – Petromyzonidae to Anotopteridae. Volume II will contain families Mirapinnidae to Xiphiidae, and Volume III Gobiidae to Linophrynidae, plus references supplemental to Clofnam and indices to common and scientific names.

The key to families usually uses one or two characters per couplet. Except at the class level, the key does not attempt to be phylogenetic and some families key out in more than one place, which should facilitate identification of polymorphic families. A useful feature is the inclusion of headings in the body of the key, e.g. Hagfishes, lampreys; Bony fishes; Pelvic fins absent; Adipose fins and photophores present. This permits the user with experience to skip the beginning of a series of couplets and arrive more quickly at an identification. Outline drawings are given for each family in the key, but there are only three text figures to illustrate key characters.

A typical family account is composed as follows. The scientific name of the family is followed by the author of the account and vernacular names in English, French, Spanish, and occasionally Russian and German. Two paragraphs follow, one giving a morphological description and the second a summary of the biology of the family. The number of world and Clofnam area genera is listed and recent revisions are cited. Keys to genera and species may be combined or separated; keys usefully give page numbers of species accounts. Figures may be included to explain characters such as location of photophores and shape of teeth.

Following a generic name heading is a short description of the genus, the number of world and Clofnam area species, common synonyms, and vernacular names. Species accounts contain Diagnosis (diagnostic characters, colour, usual and maximum size), Habitat (plus food and reproduction), and Distribution paragraphs, and some have in addition a Note which explains differences in nomenclature from Clofnam or indicates unresolved taxonomic problems.

A black and white drawing about two thirds of the page wide and a spot or shaded range map one third of the page wide accompany each species account. The drawings, supplied by the different authors, are quite variable in style, but most are of acceptable quality. The maps are small (4 cm deep) and some give spot distributions, but most use various types of hatching to describe distributions in general terms.

The taxonomic names are up-to-date although displaying a moderate conservatism. Some literature is missed. Under recent revisions for *Coregonus*, Reshetnikov (1980) is listed but G. Svardson's major study (1979. Speciation of Scandinavian *Coregonus*. Inst. Freshwater Res. Drottningholm 57: 1–95) is ignored. Common names are those adopted by FAO; these not infrequently differ from North American usage, viz. FNAM usage of starry skate and raie radiée for *Raja radiata*, instead of thorny skate and raie épineuse. It would be worthwhile to make an effort to standardize common names of widespread species.

The high standard of the contributions and editing observed in Clofnam has been maintained in FNAM. The reader is supplied with a well-organized, authoritative, and highly readable text. When the volumes are complete the reader will be able to identify 1256 species in 218 families and will have, on the average, a half-page summary of information on each species. There is a fair amount of blank space on many pages. Some of this could have been used to include more text, but perhaps that would have sacrificed esthetics. It would have been useful to have each author's contribution dated so that in a few years readers will know the period that has elapsed since literature on the taxon was summarized. Some families may have been completed several years earlier than others and are therefore less up-to-date. Modern systematics moves quickly!

The typography, page design, and matte paper are esthetically pleasing. The plasticized blue cover is water resistant. The signatures appear to be glued together rather than sewn, which does not promise well for the durability of the binding.

This is a major contribution to European and world ichthyology. The contributors, editors, and Unesco are to be congratulated, especially editors who had to ride herd over a multiplicity of personalities.

DON E. MCALLISTER AND BRIAN W. COAD

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BOOKS RECEIVED/LIVRES REÇUS

Marine Mammal Science. *Edited by J. R. Geraci. Published quarterly by the Society for Marine Mammalogy, Box 368, Lawrence, KS 66044. Vol. 1, No. 1, January 1985. 107 p. Subscription rates: $45 for U.S. libraries and nonmembers; $50 for non-U.S. ISSN 0824-0469.* This new journal publishes results of original research and observations on marine mammals, their form, function, husbandry, populations, and ecological relationships. Articles and Notes are subject to peer review; Opinions are invited Contributions on selected topics; Letters to the Editor, which will serve as a forum for communicating opinion, interpretation, and new information, will be judged on appropriateness by the Editor. Other sections in the inaugural issue are Books (reviews), News, Memories (obituaries), and Business (of the Society). The goal to produce a journal professional in appearance as well as in scientific content was reached in this first issue. [R667]

Bioeconomic Modelling and Fisheries Management. *Colin W. Clark. John Wiley & Sons, New York, NY. 1985. 291 p. $61.95 (Canada). ISBN 0-471-87394-2.* The author uses simple mathematical models as a basis for understanding the complex dynamic interactions between the biology of fish populations and the economics of the fishing industry. Major topics are methods of preventing overfishing and overcapitalization, economically effective and practical forms of regulation, management of developing fisheries, natural fluctuations of fish stocks, and complexities of marine ecosystems. The models are thoroughly analyzed and include numerous illustrations. [R668]

Artificial Reefs: Marine and Freshwater Applications. *Edited by F. M. D'Itri. Lewis Publishers, Inc., Chelsea, MI. 1985. 589 p. $49.95 (U.S.). ISBN 0-87371-010-X.* This book is the first to summarize the history and state-of-the-art of artificial fishing reef technology in marine and freshwater ecosystems. It presents information on the physical and limnological characteristics of artificial reefs, various aspects of design and construction, ecology of selected systems, and strategies to enhance benefits plus an overview of the current United States legislation and regulations. [R669]

Crustacean and Mollusk Aquaculture in the United States. *Edited by Jay V. Huner and E. Evan Brown. AVI Publishing Company, Inc., Westport, CT. 1985. 476 p. $59.00 (U.S.). ISBN 0-87055-468-9.* Specialist contributors to this book address the status of the culture of freshwater crayfishes, freshwater prawns, penaeid shrimps, homarid lobsters, oysters, marine clams, marine mussels, and abalones in the United States. Additional contributions deal with culture of miscellaneous crustaceans (e.g. soft-shelled blue crabs and spiny lobsters) and the monitoring and controlling of water quality. [R670]

Pêches du quinnat et du coho dans le détroit de Géorgie. *A. W. Argue, R. Hilborn, R. M. Peterman, M. J. Staley et C. J. Walters. Bulletin canadien des sciences halieutiques et aquatiques 211. 1985. 97 p. 8,95$ (Canada); 10,75$ (Autres pays). ISBN 0-660-91484-0; ISSN 0706-6511.* Les auteurs ont mis au point un modèle de simulation par ordinateur dans le but d'évaluer une réglementation projectée affectant pêcheurs sportif et commerciaux (saisons closes, limites de taille, limits au panier, etc.). Dans ce modèle, on maintient pour chaque classe d'âge les stocks naturels et revalorisés de saumons chinook et coho, et on évalue les divers règlements en simulant le cycle biologique de ces poissons à intervalles de deux semaines. [R671]

Liste annotée des espèces de copépodes plactoniques marine présents sur la plate-forme et la pente supérieure continentales de l'Atlantique nord-ouest (du golfe du Maine à la baie d'Ungava). *M. J. Tremblay et J. T. Anderson. Publication spéciale canadienne des sciences halieutiques et aquatiques 69. 1985. 12 p. 3,00$ (Canada); 3,60$ (Autre pays). ISBN 0-660-91487-5; ISSN 0706-649X.* Le rapport porte sur les espèces de copépodes planctoniques présentes dans les eaux de la plate-forme et de la pente continentales jusqu'à une profondeur de 1000 m, à partir des eaux intérieures du golfe du Maine jusqu'à la baie d'Ungava, y compris le golfe de Saint-Laurent et le Bonnet flamand. On présente une liste annotée des espèces, basée sur les données taxonomiques et distributionnelles publiées depuis 1969. [R672]

Incubateurs à substrat profond. *V. A. Pepper. Publication spéciale canadienne des sciences halieutiques et aquatiques 71. 1985. 27 p. 4$ (Canada); 4,80$ (Autre pays). ISBN 0-660-91488-3; ISSN 0706-649X.* On trouvera dans le présent guide une description des méthodes utilisées dans l'exécution d'un projet de mise en valeur du saumon de l'Atlantique (*Salmo salar*), nécessitant l'incubation des œufs pour assurer des apports de saumoneaux destinés à des fins de repeuplement. [R673]

The Early Atlantic Salmon Fishery in Newfoundland and Labrador. *V. R. Taylor. Canadian Special Publication of Fisheries and Aquatic Sciences 76. 1985. 71 p. $6.00 (Canada); $7.20 (Other countries). ISBN 0-660-11792-4; ISSN 0706-6481.* This report examines the salmon fishery of insular Newfoundland and of Labrador from earliest times to the beginnings of statistical records in 1910. The study relates major variations in the numerical record to social and economic upheavals and to major trends in apparent abundance of salmon. [R674]

ANNOUNCEMENTS/AVIS

NATO Advanced Research Workshop on Numerical Ecology

An Advanced Research Workshop on Numerical Ecology, sponsored by NATO Scientific Affairs Division, will be held in Roscoff (Brittany, France) from June 2 (arrival) to June 12 (departure), 1986. Forty community ecologists, specialists in statistics, will be given the opportunity to explore recent developments in mathematics; these will be presented during the morning sessions by mathematicians and methodologists. Subjects will include fractal theory, scaling techniques, non-linear multivariate analysis with optimal scaling, clustering with models, spatial analysis, and qualitative path analysis. Working groups will meet during the afternoons to examine how to apply these mathematical methods to the study of ecosystems. The working groups will focus on (1) microorganisms, (2) benthic communities, (3) pelagic communities, (4) dynamic biological oceanography and limnology, (5) terrestrial vegetation, and (6) terrestrial fauna. Participation will be mostly by invitation.

Besides the list of invited participants, community ecologists who are trained in multivariate statistics or data analysis are invited to apply. A limited number of participants will be selected by the International Organising Committee on the basis of (1) their potential contribution to the success of the Workshop (judgment based on the list and pertinence of their recent publications), (2) the distribution by countries of origin, and (3) the distribution by fields of specialty, given the six working groups listed above. Application files should contain information on these three points; microbial community ecologists are especially invited to apply. A few self-supported observers can be accepted for the morning sessions. Applications should be sent before December 20, 1985, to Dr. Pierre Legendre, Département de Sciences biologiques, Université de Montréal, C.P. 6128, Succursale A, Montréal (Qué.), Canada H3C 3J7.

Réunion de travail en écologie numérique (OTAN)

Un groupe de travail en écologie numérique se réunira à Roscoff (Bretagne, France) du 2 juin (arrivée) au 12 juin 1986 (départ), sous les auspices de la Division des Affaires scientifiques de l'OTAN. Quarante écologistes des communautés, spécialisés en analyse des données, auront ainsi l'occasion d'étudier certaines nouvelles méthodes mathématiques. Celles-ci seront présentées au cours des matinées par des mathématiciens et des méthodologistes. Parmi les sujets traités, citons la théorie des fractals, les méthodes d'ordination, l'analyse multidimensionnelle non-linéaire basée sur le calcul d'échelles optimales, le groupement suivant différents modèles écologiques, l'analyse spatiale, ainsi que l'analyse des coefficients de cheminement lorsque le modèle inclut des variables qualitatives. Les groupes de travail se réuniront, au cours des après-midis, pour étudier comment ces méthodes mathématiques peuvent être employées à l'étude des écosystèmes. Les groupes de travail suivants sont prévus : (1) microorganismes, (2) communautés benthiques, (3) communautés pélagiques, (4) océanographie et limnologie biologiques dynamiques, (5) végétation terrestre et (6) faune terrestre. La plupart des participants viendront sur invitation.

En plus des participants invités, les écologistes des communautés qui possèdent une bonne formation en statistique multidimensionnelle ou en analyse des données sont invités à soumettre leur candidature. Un nombre restreint de participants non-invités seront retenus par le Comité international d'Organisation, à l'aide des critères suivants : (1) leur contribution possible au succès de la réunion (jugement fondé sur la liste ainsi que la pertinence de leurs publications récentes), (2) la répartition par pays d'origine et (3) la répartition des participants parmi les six groupes de travail mentionnés ci-dessus. Quelques observateurs auto-financés pourront aussi être acceptés pour les sessions du matin. Les dossiers de candidature devront comporter des informations quant à ces trois points; les écologistes des communautés microbiennes sont spécialement invités. Tous les dossiers de candidature devront être parvenus avant le 20 décembre 1985 au Dr Pierre Legendre, Département de Sciences biologiques, Université de Montréal, C.P. 6128, Succursale A, Montréal (Qué.), Canada H3C 3J7.

First Call for Papers

American Fisheries Society 1986 Annual Meeting

The American Fisheries Society will hold its 1986 annual meeting with the International Association of Fish and Wildlife Agencies from September 14 to 18, 1986, in Providence, Rhode Island. The meeting will feature symposia, contributed paper sessions, poster sessions, social events, and business meetings.

Proposals for symposia or organized contributed paper sessions are invited. Proposals (including a brief justification,

Première demande de la présentation de documents

Réunion annuelle de l'American Fisheries Society — 1986

L'American Fisheries Society tiendra sa réunion annuelle de 1986, de concert avec l'International Association of Fish and Wildlife Agencies, du 14 au 18 septembre 1986 à Providence (Rhode Island). Colloques, séances de présentation et d'affichage de documents, événements sociaux et réunions d'affaires sont prévus pour l'occasion.

Nous vous invitons à soumettre des propositions en vue des

speakers' names, paper titles and abstracts (see instructions below), time required, and section sponsorship, if any) for these special sessions must be received by December 1, 1985. Preference will be given to symposia that are tightly structured, evaluate specific hypotheses, and contribute to our understanding of fishery science.

Titles and abstracts for poster and contributed paper sessions also are solicited. These abstracts must be received no later than January 15, 1986. Abstracts (not to exceed 200 words) should be informative, rather than indicative, and must be typed (double spaced) on a single sheet of paper and include in order (1) a succinct, descriptive title, (2) the full name, address, and telephone number of each author (with speaker underlined), and (3) a text that provides objectives, principal results, and conclusions. In addition, the following information should be typed at the bottom of the page: (1) your preference for poster or contributed paper session, (2) your projection needs, if any, (3) the mailing address to which correspondence is to be sent, and (4) whether you are a student (i.e. a student at the time of abstract submission). Papers should report on recent research that has not been published and must include new, specific information. Papers accepted for the program will be scheduled by subject area.

The original and six copies of the *abstract* must be submitted for review by the Program Committee. If authors wish to be notified of the receipt of their abstract, and, subsequently, of its acceptance, they should enclose two self-addressed and STAMPED post cards (9 × 12.5 cm) bearing the title of the paper and the author's name. One will be returned upon receipt of the abstract; the second will be returned when a decision as to acceptance is made.

All special-session proposals, abstracts, and questions regarding the program should be directed to Roy A. Stein, 1986 Program Chair, Department of Zoology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA, telephone (614) 422-7826 or 422-8088. Questions regarding facilities and meeting arrangements should be directed to Richard T. Sisson, 1986 Arrangements Chair, Department of Environmental Management, Division of Fish and Wildlife, Government Center, Tower Hill Road, Wakefield, RI 02879, USA, telephone (401) 789-3094.

colloques. Les propositions (donner une courte justification, les noms des conférenciers, les titres des documents et les résumés (voir instructions ci-dessous) et indiquer le temps et le parrainage s'il y a lieu) concernant ces séances spéciales doivent nous parvenir d'ici le 1 décembre 1985. La priorité sera accordée aux propositions bien structurées, qui permettent d'évaluer des hypothèses précises et qui favorisent notre compréhension des sciences halieutiques.

Nous vous demandons également de soumettre les titres et les résumés des documents qui seront présentés et affichés. Ces résumés doivent nous parvenir au plus tard le 15 janvier 1986. D'un maximum de 200 mots, ils doivent être de caractère informatif et non seulement indicatif, être dactylographiés (à double interligne) sur une seule page et inclure, dans l'ordre, (1) un titre succinct et descriptif, (2) le nom complet, l'adresse et le numéro de téléphone de chaque auteur (avec le nom du conférencier souligné) et (3) un texte établissant les objectifs, les principaux résultats et les conclusions. En outre, les renseignements suivants doivent être tapés au bas de la page: (1) votre choix entre une séance d'affichage ou sur une séance de présentation de documents, (2) vos besoins en matière de projection s'il y a lieu, (3) l'adresse à laquelle votre correspondance doit être envoyée et (4) une indication de status d'étudiant, s'il y a lieu (c'est-à-dire, étudiant lors de la présentation du résumé). Les documents doivent porter sur des recherches récentes qui n'ont pas été publiées et doivent inclure des données nouvelles et précises. Les documents acceptés seront classés par sujet.

La version originale, de même que les six exemplaires du *résumé*, doivent être soumis au Comité responsable du programme. Si les auteurs veulent recevoir un accusé de réception de leur résumé et de l'acceptation de ce dernier le cas échéant, ils doivent inclure deux cartes préadressées et AFFRANCHIES (9 sur 12,5 cm) portant le titre du document et le nom de l'auteur. L'une sera renvoyée dès réception du résumé, l'autre lorsqu'une décision au sujet de son acceptation sera prise.

Tous les résumés et propositions ayant trait à des séances spéciales et toute question relative au programme doivent être soumis à Roy A. Stein, 1986 Program Chair, Department of Zoology, The Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA, téléphone (614) 422-7826 ou 422-8088. Les questions portant sur les services et les dispositions à prendre doivent être présentées à Richard T. Sisson, 1986 Arrangements Chair, Department of Environmental Management, Division of Fish and Wildlife, Government Center, Tower Hill Road, Wakefield, RI 02879, USA, téléphone (401) 789-3094.

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Articles

Estimating the standing biomass of aquatic macrophytes **J. A. Downing and M. A. Anderson**

Interactive effects of cadmium, polychlorinated biphenyls, and fuel oil on experimentally exposed English sole (*Parophrys vetulus*) **L. Rhodes, E. Casillas, B. McKnight, W. Grolund, M. Myers, P. Olson, and B. McCain**

Morphological abnormalities in Chironomidae (Diptera) larvae as measures of toxic stress in freshwater ecosystems: indexing antennal deformities in *Chironomus* Meigen **W. F. Warwick**

Foraging behavior of hatchery-produced coho salmon (*Oncorhynchus kisutch*) smolts on live prey **C. A. Paszkowski and B. L. Olla**

Effects of successive flow perturbations on stream invertebrates **J. R. Irvine**

Influence of mesh composition, velocity, and run time on the catch and length composition of juvenile alewives (*Alosa pseudoharengus*) and blueback herring (*A. aestivalis*) collected by pushnet **B. M. Jessop**

Biological control of excessive phytoplankton growth and the enhancement of aquacultural production **D. W. Smith**

Effects of manipulations of aluminum concentrations and pH on phosphate uptake and photosynthesis of planktonic communities in two Precambrian Shield lakes **C. Nalewajko and B. Paul**

Fertilization and egg retention in artificially inseminated female American lobsters, *Homarus americanus* **S. L. Waddy and D. E. Aiken**

Potential of parasites for use as biological indicators of migration, feeding, and spawning behavior of northwestern Atlantic herring (*Clupea harengus*) **S. E. McGladdery and M. D. B. Burt**

Variabilité interannuelle d'un indice d'intensité des remontées d'eau dans le secteur du cap Blanc (Mauritanie) **R. Arfi**

Influence of snowcover development and ground freezing on cation loss from a wetland watershed during spring runoff **D. C. Pierson and C. H. Taylor**

Potential usefulness of chlorine for controlling Pacific salmon leeches, *Piscicola salmositica*, in hatcheries **S. M. Bower, L. Margolis, and R. J. MacKay**

Comparative analysis of ionoregulation in rainbow trout (*Salmo gairdneri*) of different sizes following rapid and slow salinity adaptation **C. E. Johnston and J. C. Cheverie**

Physiological response of juvenile rainbow trout, *Salmo gairdneri*, to acid and aluminum — prediction of field responses from laboratory data **C. M. Neville**

Variation in body morphology among British Columbia populations of coho salmon, *Oncorhynchus kisutch* **E. B. Taylor and J. D. McPhail**

Variation in burst and prolonged swimming performance among British Columbia populations of coho salmon, *Oncorhynchus kisutch* **E. B. Taylor and J. D. McPhail**

Reviews/Synthèses

Acidification and toxicity of metals to aquatic biota **P. G. C. Campbell and P. M. Stokes**

Notes

The right to fish as an external benefit of open access **R. E. Townsend**

Changes to Stock Reduction Analysis indicated by Schnute's general theory **D. K. Kimura**

Removal of follicle wall components from ovarian oocytes of the brook trout, *Salvelinus fontinalis* **C. A. Lessman, S. Kavumpurath, and H. R. Habibi**

Perspectives

American lobster, green sea urchin, and seaweeds: a reappraisal **R. J. Miller**

Book reviews/Comptes rendus

QSAR in environmental toxicology (edited by L. E. Kaiser) **D. J. Ecobichon**
Proceedings of the National Aquaculture Conference. Strategies for aquaculture development in Canada (edited by G. I. Pritchard) **J. Hynes and A. Castledine**
Storm surges — meteorological ocean tides (T. S. Murty) **P. F. Hamblin**
Communication and behavior of whales (edited by Roger Payne) **E. Mitchell and R. R. Reeves**

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Some Recent Articles
Kl. Mechsner: The Influence of Seasonal Light Variations on the Growth of *Sphaerotilus natans.* **N. D. Yan and W. Geiling:** Elevated Planktonic Rotifer Biomass in Acidified Metal-contaminated Lakes near Sudbury, Ontario. **T. Tirén and K. Petterson:** The Influence of Nitrate on the Phosphorus Flux to and from Oxygen Depleted Lake Sediments. **J. Moreau** *et al:* A Model for Rapid Growth Evaluation in Fishes: The Case of the Cyprinids of Some Large French Rivers. **J. L. Bagliniere and D. Arribe-Moutounet:** Microrépartition des populations de truite commune (*Salmo trutta* L.) de juvenile de saumon atlantique (*Salmo salar* L.) et des autres espèces présentes dans la partie haute du Scorff (Bretagne). **P. Leentvaar:** Alto Sinú Hydroelectric Project in Columbia: Possible Consequences for the Environment. **K. Papińska:** Carnivorous and Detritivorous Feeding of *Mesocyclops leuckarti* Claus (Cyclopoida, Copepoda). **W. Decraemer:** Revision and Phylogenetic Systematics of the Desmoscolecida (Nematoda).

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Book reviews/Comptes rendus

Fishes of the north-eastern Atlantic and the Mediterranean/Poissons de l'Atlantique nord-est et de la Méditerranée (edited by P. J. P. Whitehead, M.-L. Bauchot, J.-C. Hureau, J. Nielsen, and E. Tortonese) D. E. McAllister and B. W. Coad **1852**

Books received/Livres reçus **1853**
Announcements/Avis **1854–1855**
Coming next month/À paraître le mois prochain **1856–1857**

Canadian Journal of Fisheries and Aquatic Sciences

Journal canadien des sciences halieutiques et aquatiques

Volume 42, No. 11, November 1985

Volume 42, nº 11, novembre 1985

Articles

Identification of sockeye salmon (*Oncorhynchus nerka*) stocks in the Stikine River based on egg size measurements P. C. Craig **1696–1701**

Patterns of aggressive attack in juvenile steelhead trout (*Salmo gairdneri*) J. C. Abbott and L. M. Dill **1702–1706**

Partitioning light attenuation in an acidic lake S. W. Effler, G. C. Schafran, and C. T. Driscoll **1707–1711**

Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan Lake system, Nevada M. L. F. Estep and S. Vigg **1712–1719**

Prey selection by freshwater predators with different foraging strategies S. D. Cooper, D. W. Smith, and J. R. Bence **1720–1732**

Growth and production of *Eogammarus confervicolus* (Amphipoda: Anisogammaridae) at a log storage site and in areas of undisturbed habitat within the Squamish estuary, British Columbia M. J. Stanhope and C. D. Levings **1733–1740**

Aluminum bioaccumulation and toxicity to *Daphnia magna* in soft water at low pH M. Havas **1741–1748**

Inter- and intra-specific variability in the response of zooplankton to acid stress E. E. Price and M. C. Swift **1749–1754**

Effect of female size, egg size, and water temperature on developmental biology of chum salmon (*Oncorhynchus keta*) from the Nitinat River, British Columbia T. D. Beacham and C. B. Murray **1755–1765**

Equilibrium yields and yield isopleths from a general age-structured model of harvested populations T. A. Lawson and R. Hilborn **1766–1771**

Fish hepatocytes: a model metabolic system T. W. Moon, P. J. Walsh, and T. P. Mommsen **1772–1782**

Selenium requirement of a bloom-forming planktonic alga from softwater and acidified lakes J. D. Wehr and L. M. Brown **1783–1788**

Spring meltwater mixing in small arctic lakes M. A. Bergmann and H. E. Welch **1789–1798**

Annulus development on the second dorsal spine of the spiny dogfish (*Squalus acanthias*) and its validity for age determination R. J. Beamish and G. A. McFarlane **1799–1805**

Optimizing sampling design for herring spawn surveys in the Strait of Georgia, B.C. J. F. Schweigert, C. W. Haegele, and M. Stocker **1806–1814**

Suitability of vertical slot and Denil fishways for passing north-temperate, nonsalmonid fish K. Schwalme, W. C. Mackay, and D. Lindner **1815–1822**

Distinguishing between one-day-old cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) eggs by gas chromatography and SIMCA pattern recognition H. Knutsen, E. Moksness, and N. B. Vogt **1823–1826**

Seasonal patterns in lipid content of Lake Michigan macroinvertebrates W. S. Gardner, T. F. Nalepa, W. A. Frez, E. A. Cichocki, and P. F. Landrum **1827–1832**

Notes

Simplified calculation of optimum spawning stock size from Ricker's stock recruitment curve R. Hilborn **1833–1834**

Use of visual census for estimating population size in northern pike (*Esox lucius*) L. J. Turner and W. C. Mackay **1835–1840**

Perspectives

Reciprocal oscillations in densities of larval fish and potential predators: a reflection of present or past predation? K. T. Frank and W. C. Leggett **1841–1849**

Obituary/Nécrologie

Henry Benedict Hachey, 1901–1985 N. J. Campbell **1850–1851**

(*continued inside back*)

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